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Comparative 3D structural ornamentations on the eggs of *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) of Burdwan, West Bengal, India

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ABSTRACT: *Aedes aegypti* and *Aedes albopictus* are potential arboviral vectors that are responsible for spread of dengue worldwide. Studies of these vectors and their bionomics form an important part in the vector controlling strategy. In the present piece of work, efforts have been made to differentiate between the eggs of these two species morphologically through scanning electron microscope. From the scanning electron micrographs of both of the species morphological differences were very clear. The eggs of *Aedes albopictus* were found to be much smaller in structure than that of *Aedes aegypti*. Moreover the micropylar apparatus, extrachorionic structure were also significantly different. Various species can be differentiated by viewing the scanning electron micrographs of the eggs. Stereomicroscopic structures are essentially useful in determining the difference between the species. The various differences in egg structure might be due to the environmental parameters they are laid at.

KEYWORDS: *Aedes albopictus, Aedes aegypti,* Micropyle, Scanning electron microscopy, Tubercle

INTRODUCTION

The global resurgence and prevalence of vector borne disease such as dengue has generated an awakening for awareness. The vectors responsible for this arbovirus are *Aedes aegypti* (Linn.) and *Aedes albopictus* (Scuse) which have attracted multiple research fields and prompted scientists and researchers to have a wider look in these mosquito species. *Ae. albopictus* is an adaptive and invasive species co-existing with or displacing *Ae. aegypti* in different regions (Paupy et al.,2009) . Studies related to these species include vector competence (Boromisa et

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al., 1987; Diallo et al., 2008; Moore et al., 2007), insecticide resistance (Hidayati et al.,2005; Stasiak, 1969; Wesson, 1990) spatial, temporal and geographical analyses (Benedict et al.,2007; Castro Gomes et al.,2005; Francy et al.,1990), and ecological and evolutionary studies (Juliano et al.,2002; Pumpuni et al.,1992). Scanning electron microscopic studies are one of the most important studies related to the characterization of these species. Scanning electron microscopy (SEM) evaluation differs from transmission electron microscopy (TEM) in that the whole specimen can be viewed. In an effort to contribute to the knowledge about *Aedes* sp, it is necessary to highlight the egg morphology too. SEM reveals the 3D ultrastructural details of the egg which cannot be achieved by the traditional light microscope. Though there are a number of studies regarding the egg of *Aedes aegypti* (Sasa et al., 1971; Matsuo et al; Moriya et al., 1973) only scanty literature is available on the comparative anatomical analysis of the eggs of *Aedes aegypti* and *Aedes albopictus* prevalent in West Bengal. The present piece of work deals with the comparative 3D surface topography of the eggs of *Aedes aegypti* and *Aedes albopictus* from Burdwan, West Bengal.

MATERIALS AND METHODS

Collection of eggs: *Aedes albopictus* and *Aedes aegypti* mosquitoes has been hatched, reared, maintained and cultured for several generations in the mosquito insectary of the Parasitology and Microbiology Research laboratory, Zoology Department, The University of Burdwan. All the mosquitoes were maintained in 25±2ºC, 75±5% relative humidity and 12:12 h (light:dark) photoperiod in the insectary (Deng et al., 2012) where the cages measured 30 cm x 30 cm x 30 cm. 10% sucrose solution soaked in cotton pad was given prior to blood feeding. The eggs were laid on a moist filter paper and allowed to incubate in this moisture. Few eggs prior to incubation were collected for Scanning Electron Microscopy evaluation.

Scanning electron microscopy: Eggs were fixed in 2.5% glutaraldehyde (HIMEDIA) in phosphate buffer (PBS) at pH 7.4 at 4ºC for 45 mins and thereafter washed in PBS giving two changes of 10 mins each, followed by post fixation in osmium tetroxide (HIMEDIA) for 1 hr at room temperature (Choochote et al., 2001). The eggs were then dehydrated by passing through an ascending series of ethanol (MERCK); 50%, 70%, 90% and 100% (10 mins each). Eggs were then immersed for 5-7 mins in 1:1 ratio of absolute alcohol and isoamyl acetate(HIMEDIA) and then in pure isoamyl acetate (HIMEDIA) for 5-7 mins again and dried by the critical point drier (HCP-2, Tokyo, Japan), mounted on stubs by just placing them directly on stubs and gold coated in an ion sputter (IB -2 Ion Coater, EICO Engineering, Japan) and viewed by the Hitachi S-150 scanning microscope and micrographs were taken.

RESULTS AND DISCUSSION

Comparisons of the two species' eggs depict evident distinctions between them. The terminology followed here is of Harbach and Knight (1980). Out of the various attributes like egg dimensions, micropylar apparatus, tubercle type, chorionic structure etc studied, these species' eggs were found to be only 48.48% different from each other (Suman et al., 2011). Eggs of *Aedes albopictus* were found to be much smaller in structure than that of *Aedes*

Figure 1: Scanning electron micrograph of *Aedes aegypti* (a) Entire egg length

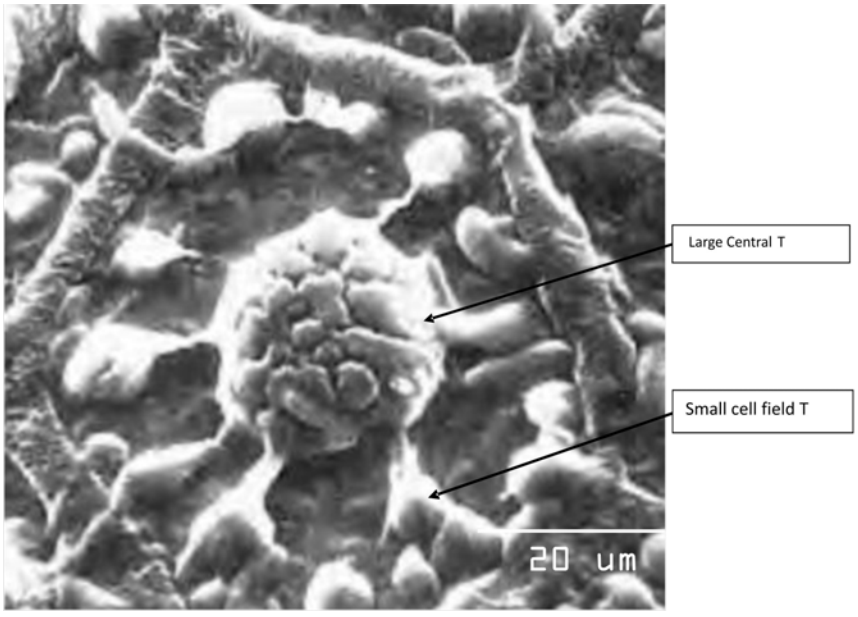

Figure 1: Scanning electron micrograph of *Aedes aegypti* (b) a single reticulum showing the central tubercle. T= Tubercle.

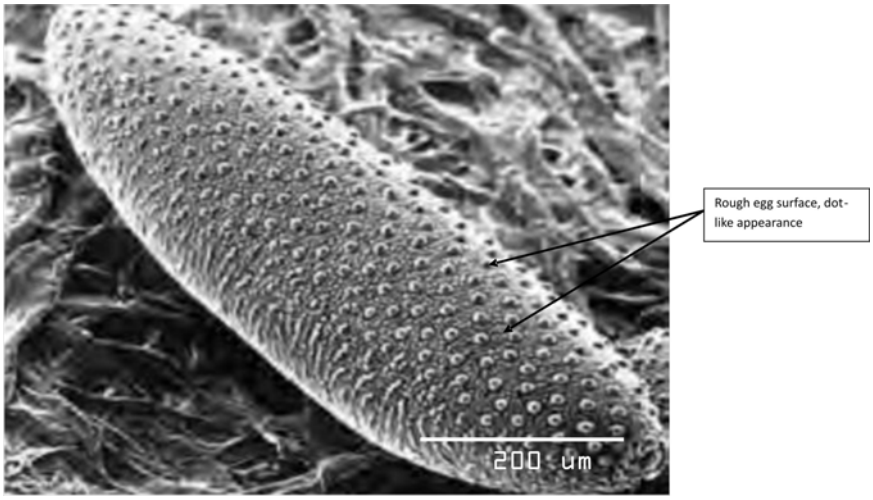

Figure2: Scanning electron micrograph of *Aedes albopictus* (a) An entire egg length.

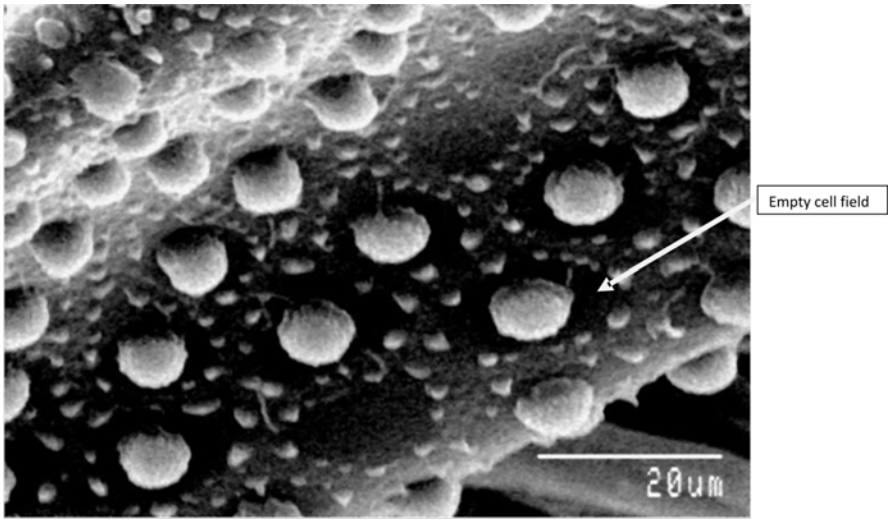

Figure2: Scanning electron micrograph of *Aedes albopictus* (b)dorsal view showing empty cell field.

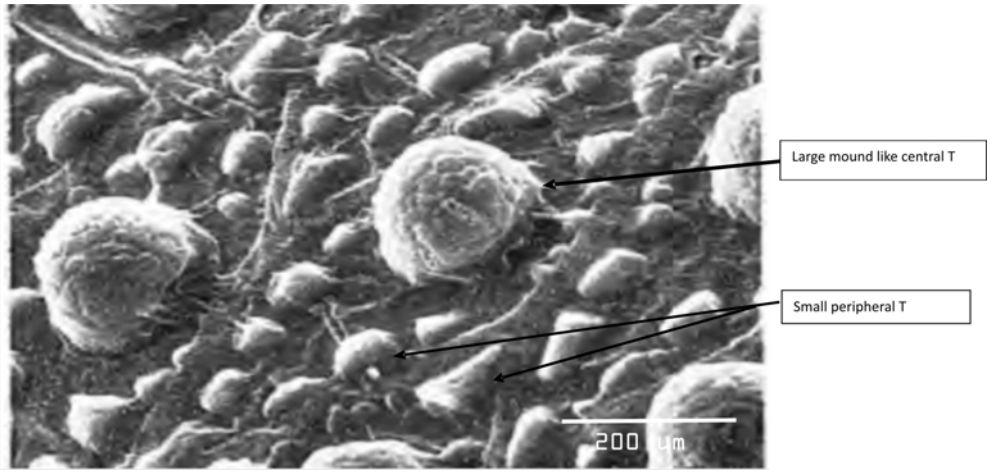

Figure2: Scanning electron micrograph of *Aedes albopictus* (c) large swollen tubercle.T= Tubercle.

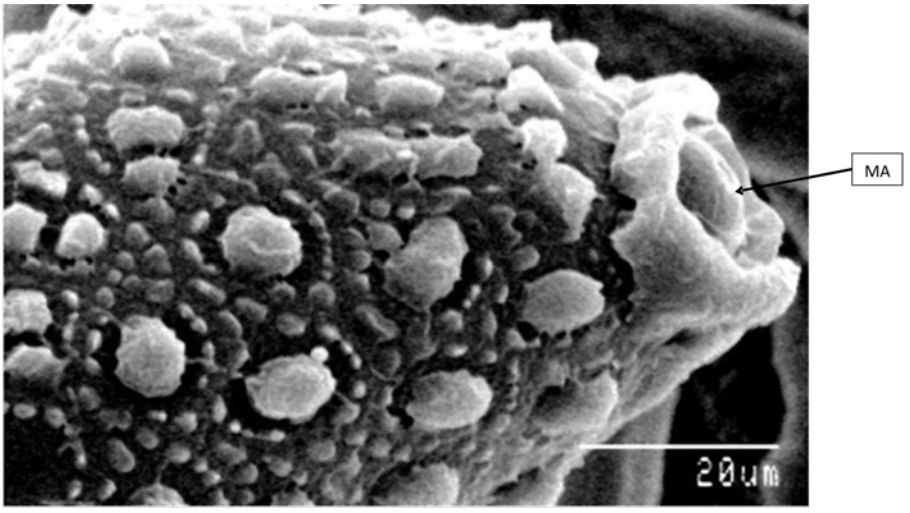

Figure2: Scanning electron micrograph of *Aedes albopictus* (d) micropylar apparatus (MA)

aegypti, and were more tapered cylindrically at the posterior end, whereas the eggs of *Aedes aegypti* showed much wider posterior side. Both species' eggs were shiny, pitch black in outlook and looked rice-like when laid. The egg surface was found to be rough in case of both the species', but the tubercles looked evenly placed in the micrographs in case of *Aedes albopictus* (Fig 2a) and irregularly placed with distinct gaps between each tubercle in case of *Aedes aegypti* (Fig 1a).

The outer chorionic cell field is the space between the hexagonal or polygonal boundary. It is the space where the tubercle lies centrally. The boundary guarding the cell field is known as "outer chorionic reticulum". In this work the ventral chorionic structure has been highlighted. In case of *Aedes albopictus*, the outer chorionic reticulum was mostly hexagonal (Fig 2b), with very few pentagonal structures. Within these polygons tubercles were present, which again differ from species to species and act as prominent species identification marker. *Aedes albopictus* eggs showed to have a large central tubercle (Fig 2c), swollen mound-like and a bit protruding with a slight dent in the middle; whereas eggs of *Aedes aegypti* also showed the same but often two tubercles were seen to be present in the same reticulum in the same cell field (Fig 1b). The cell field was seen to be completely empty in case of *Aedes albopictus* with smaller peripheral tubercles arranged in the outer chorionic reticulum (Fig 2 b-d), but cell field failed to be empty in case of *Aedes aegypti*. Smaller tubercles were often found to be in connection with the large central tubercle (Fig 1b). The collar of the micropylar apparatus of the *Aedes albopictus* was seen to be circular without any sectors and the micropyle was seen to be inserted into a shallow groove-like structure (Fig 2d); however the collar of the micropylar apparatus of *Aedes aegypti* had sectors.

Scanning electron microscopy provides a greater depth into the fine ornamentations of the eggs which enable to distinguish between various species. Though SEM structures of *Aedes albopictus* and *Aedes aegypti* are hard to differentiate, there are still certain features that bring out the difference between the species. Very little work has been done on the scanning electron microscopy of *Aedes* sp eggs. The shiny black colour of the *Aedes* eggs is thought to be mainly due to the darkening of the endochorion after the eggs are laid (Hinton and Service, 1969). Though the function of the exochorion or the outer layer of the *Aedes* eggs is not properly understood, Hinton and Service (1969) reported that in other species like *Culex* it holds a thin film of air. The outer egg shell of the aedine eggs is roughly polygonal but often hexagonal. The shapes of polygons differ from species to species and that is a remarkable distinguishing feature of identification (Hinton and Service, 1969). In *Aedes lineatopennis*, the micropylar collar were found to be fragmented and the exochorion reticulum was irregular (Choochote et al., 2001), which was a distinguishing feature specific to this species only and differed from the other *Aedes* species. The findings of Linley (1989) agreed with our study with respect to the length of the eggs, which stated that eggs of *Aedes aegypti* are longer than eggs of *Aedes albopictus*. Similar studies by the same author showed eggs of *Aedes bahamensis* to be significantly longer and larger than the two species studied in our work. The micropylar collar of *Aedes bahamensis* was not seen to be prominent but discontinuous, while those of *Aedes aegypti* were prominent. *Aedes albopictus* showed the same feature as

Aedes bahamensis (Linley, 1989). According to Suman et al. (2011) the strong solid wall like exochorions of *Aedes albopictus* might be responsible for their protection from dessication when laid in containers, whereas exochorions of *Aedes aegypti* were found to be reticulated and interwoven. Nevertheless, the present work correlated with the findings done in the past.

From the present study, minute differences in the egg ornamentations were easily distinguished through SEM and hence can be used as a relevant tool to identify the differences in species. Stereomicroscopic structures are essentially useful in determining the difference between the species. The differences in the architecture of the egg structure of the species may be adapted to the environment and their habitat.

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Seasonal variations in the prevalence and intensities of infestation of phthirapteran ectoparasites of domestic fowl

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ABSTRACT: In the present paper an attempt has been made to observe the impact of four ecofactors (RH, Temperature, Heat index and Photoperiod) on the prevalence and infestation intensity of five phthirapteran species infesting domestic fowl (*Lipeurus caponis, Lipeurus tropicalis, Menopon gallinae, Goniocotis gallinae, Goniodes dissimilis*). By and large the ecofactor taken into consideration did not have significant impact on the prevalence. However the RH and photoperiod appeared to have influence on intensities of *L. caponis* and *L. tropicalis* which prefer wing feathers. On the other hand temperature and heat index seem to have influence on intensities of *G. gallinae*, *G. dissimilis* and *M. gallinae* which generally prefer body feathers of host bird.

Key words: Phthiraptera, Ischnoceran, Amblyceran, Infestation intensity, Prevalence.

INTRODUCTION

In spite of the fact that pthirapteran ectoparasites live in a microclimate of considerable constancy (made up of feathers / hair), they are not able to escape from the climax of seasonal changes, selected workers have noted the seasonal variations in the populations of phthiraptera infesting few host birds Foster (1969), Agarwal and Saxena (1979), Chandra *et al*., 1990, Clark *et al*., 1994, Srivastava *et al*., 2003). Workers like Marshall (1981) and Price and Graham (1997) have made attempts to review the work done on this aspect. In the present study an attempt has been made in the different months of the year to record the variations in

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the mean monthly prevalence and mean monthly intensity of five phthirapteran species infesting domestic fowl in district Rampur and adjoining areas.

MATERIALS AND METHODS

One hundred domestic fowls were deloused (Fumigation method) every month during the year 2012 in five localities (Rampur proper, Swar, Tanda, Bilaspur and Rudrapur) of district Rampur and adjoining areas. The louse load obtained from every bird was placed in separately labeled vials and was separated according to species. The mean monthly prevalence and intensity of infestation was computed every month throughout the year. At the same time mean monthly temperature, relative humidity, photoperiod and heat index was also taken into consideration. Finally, attempts were made to record the degree of correlation between the mean monthly prevalence (as well as mean monthly intensity) and the four aforesaid eco-factors (mean monthly temperature, relative humidity, photoperiod and heat index).

RESULTS

In different months of year from January to December, the prevalence and mean monthly intensities of five different phthirapteran ectoparasite on domestic fowls of district Rampur and adjoining areas during the year 2012 is indicated in table 1. The values of Karl Pearson's Coefficient of Correlation (r) between prevalence, mean monthly infestation intensities of five phthirapteran ectoparasite and the mean monthly ecofactors have been discussed below-

Lipeurus tropicalis: Moderate correlation existed between prevalence of *Lipeurus tropicalis* and R.H, heat index and photoperiod (r = 0.41, 0.41 and -0.46, respectively). However strong positive correlation existed between prevalence of *Lipeurus tropicalis* and heat index (r = 0.56) (Table 2). Strong positive (significant) correlation existed between infestation intensity of *L. tropicalis* and RH, temperature as well as heat index (r = 0.64, 0.60 and 0.67, respectively), The value of correlation between infestation intensity and photoperiod remained, 0.51, (Table 2).

Menopon gallinae: Moderate correlation existed between prevalence of *Menopon gallinae* and R.H as well as photoperiod (r = -0.56 and 0.48. However correlation with prevalence and temperature as well as heat index remained negligible (r = 0.15 and 0.12, respectively, (Table 2). Strong positive correlation existed between Intensity of infestation of *M. gallinae* and temperature as well as heat index (r = 0.64, in both cases). However correlation between Intensity of infestation of *M. gallinae* and RH as well as photoperiod remained non-significant (r = -0.29 and -0.24, respectively) (Table 2).

Goniodes dissimilis: Moderate correlation existed between the prevalence of *G. dissimilis* and RH as well as photoperiod (r = -0.55 and 0.33, respectively). However, correlation between prevalence of *G. Dissimilis* and Temperature as well as heat index was negligible (r = -0.08 and -0.11, respectively) (Table 2). Strong positive correlation existed between infestation intensity

Table 1: Prevalences and intensity of infestation of five phthirapteran species on domestic fowls during different months of the year 2012.

Species and months	Prevalence						Infestation intensity					
	Menopon gallinae	*Lipeurus caponis*	*Lipeurus tropicalis*	*Goniocotes gallinae*	*Goniodes dissimilis*	*Overall*	*Menopon gallinae*	*Lipeurus caponis*	*Lipeurus tropicalis*	*Goniocotes gallinae*	*Goniodes dissimilis*	*Overall*
January	44	39	25	39	37	**58**	42.09	49.13	5.63	30.25	27.59	**105**
February	56	38	10	51	29	**61**	98.05	65.3	19.4	31.26	28.16	**175**
March	48	39	19	41	30	**64**	69.29	38.69	9.36	32.12	32.36	**114**
April	52	34	26	40	33	**68**	100.3	137.23	14.15	43.82	53.12	**195**
May	63	52	17	53	35	**75**	179.5	191.7	41.33	61.62	63.4	**368**
June	58	46	23	51	25	**81**	187.25	261.15	65.08	115.62	93.04	**402**
July	42	37	33	31	23	**80**	127.8	34.23	103.7	84.32	23.78	**157**
August	21	23	32	28	21	**62**	101.5	23.61	62.21	92.89	23.47	**123**
September	30	19	21	14	16	**59**	72.93	35.47	123.95	49	19.75	**110**
October	17	18	18	12	15	**61**	30.58	52.82	43.94	58.33	51.66	**66**
November	15	16	21	14	16	**60**	46.8	62.3	39.2	37.1	52.6	**52**
December	18	12	13	17	13	**54**	31	43.58	41.61	52.52	36.23	**74**

Table 2: Values of Karl Pearson's Coefficient of correlation between mean monthly prevalences, mean monthly infestation intensity of five phthirapteran species and four ecofactors.

Species Name	Prevalence				Infestation intensity			
	Relative Humidity	Temperature	Heat index	Photoperiod	Relative Humidity	Temperature	Heat index	Photoperiod
Meopon gallinae	-0.56	0.149	0.124	0.485	-0.287	0.639	0.643	-0.239
Lipeurus caponis	-0.46	0.163	0.156	0.408	-0.569	0.377	0.339	0.724
Lipeurus tropicalis	0.411	0.411	0.56	-0.463	0.64	0.603	0.669	-0.513
Goniocotes gallinae	-0.514	0.05	0.033	0.44	0.334	0.652	0.695	-0.067
Goniodes dissimilis	-0.551	-0.083	-0.11	0.331	-0.538	0.288	0.24	0.788

of *G. dissimilis* and photoperiod (r = 0.79). The correlation with RH was moderate (r = -0.53). However, the correlation with temperature as well as heat index was insignificant (r = 0.29 and 0.24, respectively), (Table 2).

Lipeurus caponis: Moderate correlation 'non-significant' existed between the mean monthly prevalence and RH (r = 0.46) and photoperiod (r = 0.41). The correlation between prevalence and mean monthly temperature as well as heat index (Table 2) remained negligible (r = 0.16 each). Moderate correlation (non-significant) existed between the infestation intensity and temperature as well as heat index (r = 0.38 and 0.34). However, correlation between infestation intensity and photoperiod (r = -0.57), (Table 2) was found significant.

Goniocotes gallinae: Moderate correlation 'non-significant' existed between the prevalence of *G. gallinae* and RH as well as photoperiod (r = 0.51 and 0.44 respectively). Negligible correlation existed between the prevalence of *G. gallinae* and temperature as well as heat index (r = 0.05 and 0.03, respectively) (Table 2). Strong positive correlation existed (significant) between the intensity of infestation of *G. gallinae* and temperature as well as heat index (r = 0.65 and 0.69, respectively). Correlation with RH was moderate (r = 0.33). Negligible correlation existed between the intensity of infestation of *G. gallinae* and photoperiod (r = 0.07) (Table 2).

DISCUSSION

Phthirapteran ectoparasites reportedly exhibit seasonal variation in populations. A scrutiny of literature reveals that bird lice generally peak in summers and mammalian lice exhibit maxima during the winter months (Marshall, 1981). The factors responsible for variations in natural population of lice have been discussed from time to time and there is considerable dispute among the phthirapterists. Variety of factors reportedly affects the population levels of the avian and mammalian lice. In addition to environmental factors many host factors (viz., host grooming / preening, moulting, nesting activity, breeding and transference of lice after the hatch) and physiological factors (i.e. host hormone level) also reportedly influence the lice population. For instance, temperature reportedly causes "high intensities" in two phthirapteran species occurring on starling, *Sturnus vulgaris* (Boyd, 1951). Peak in population of one ischnoceran louse infesting house sparrows (*Passer domesticus*) has been attributed to summer temperature and photoperiod (Woodman & Dicke, 1954). Host moulting and breeding period caused "high incidence" of 6 species (in summer and spring) infesting black birds, *Turdus m. merula* (Baum, 1968). Environmental temperature and breeding habits causes marked increase in infestation of 15 species parasitizing chaffinches, robbins, black birds, blue tits and great tits (Ash, 1960). High infestations of three phthirapterans occurring on alcids during summers have been attributed to increased nesting activity and the temperature. Rise in temperature and breeding periods reportedly influence summer population build up of one amblyceran parasitizing common myna, *Acridotheres tristis* (Chandra *et al.*, 1990). Foster (1969) suspected that the breeding time of two haematophagous species (*Ricinus picturatus* and *Menacanthus sp.*) might be controlled by the reproductive hormones of the host bird, orange crowned warbler (*Vermivora cellata*). However, while working on the population of three lice infesting

common myna, *Acridotheres tristis* (Srivastava, 2003) concluded that in case of haematophagous *Menacanthus eurysternus* the effect of testicular weight on egg index of lice can be quite high but the ovarian weight appeared to have negligible effect. They further concluded that it is quite unlikely that the gonadal hormone would influence the reproductive potential of lice on male bird and not on female birds.

The analysis of correlation between the prevalence of five poultry lice and the eco-factors indicated that none of the eco-factors (viz. temperature, relative humidity, heat index and photoperiod) taken into consideration had any significant impact. On occasional instances moderate correlation existed between the prevalence of lice and RH, temperature, heat index and photoperiod but the values of correlation coefficient were not found statistically significant. As the prevalence of lice is dependent on transmission by direct contact among lousy and louse free birds. On the other hand, significant correlation existed between the mean intensity of infestation of *L. caponis* and RH and photoperiod. In case of *L. tropicalis* significant correlation existed between RH, temperature and heat index (correlation with photoperiod was moderate). In case of poultry fluff louse, *G. gallinae* significant correlation existed between intensity of infestation and temperature as well as heat index (correlation with RH and photoperiod were found non-significant). In case of *G. dissimilis*, significant correlation existed between intensity of infestation and photoperiod only (correlation with RH was moderate). Lastly in case of poultry shaft louse, *M. gallinae*, significant correlation existed between intensity of infestation and heat index (correlation between RH and photoperiod were non-significant).Thus, the aforesaid results indicate that in case of *L. caponis* (poultry wing louse) and *L. tropicalis (*also prefers wings), the RH and photoperiod may have influence on the population build up (since both are frequently exposed to the outer atmosphere). On the other hand, *G. gallinae*, *G. dissimilis* and *M. gallinae* (which typically reside inside body feathers and appear to prefer high temperature zone offered inside the plumage by the host. The present studies provides preliminary clue regarding the impact of environmental factors on the population levels of different poultry lice, the temperature and heat index may promote the population build up.

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Forest vegetation types related variation in the diversity and community structure of dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) in the moist south Western Ghats

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ABSTRACT: Assessment of diversity and community structure of dung beetles in the shola, evergreen and deciduous forests of Periyar Tiger Reserve (PTR) of the South Western Ghats, south India, revealed that vegetational differences affect the dung beetle population, and abundance and species richness of dung beetles was highest in the evergreen forests. High similarity in species composition between evergreen and shola forests was recorded. No vegetation specific indicator species was recorded with *IndVal* analysis from the three forest types, indicating that PTR is a habitat under stress. Four detector species were recorded and monitoring the detector species would enable in understanding the future direction of change in the various vegetation types in PTR.

KEY WORDS: Dung beetles, Vegetation types, the south Western Ghats

INTRODUCTION

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) are an important group of primary decomposers in the forest ecosystem. They play an important role in the ecosystem by aiding in the recycling of Nitrogen and other nutrients, removing dung from soil surface, protecting seeds from predation, seed dispersal, soil conditioning as primary agents in soil aeration and reducing populations of disease-causing organisms such as hookworms (Hanski, 1991). Dung beetles are affected by the structure of vegetation, which is a main factor determining the organization of dung beetle communities in tropical rainforests (Scheffler, 2005). A change in

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vegetational cover can lead to differences in mammalian fauna which, in turn, affect dung beetle populations (Estrada *et al*., 1999). Very few studies have addressed the ecology and community structure of the forest dung beetles in the Western Ghats (Sabu *et al*., 2006; Vinod and Sabu, 2007; Vinod, 2009) and no data exists on the forest vegetation type specific variation on the community structure of dung beetles from the region. In the present study, dung beetle fauna in different vegetation types in the moist western slopes of the south Western Ghats is analysed.

MATERIALS AND METHODS

The study was carried out in the Periyar Tiger Reserve (9^0 15' N - 9^0 40' N; 76^0 55'E - 77^0 25' E; 800 to 1200 m asl, 777 km^2), Thekkady, located in the southern Western Ghats of Kerala state. Annual rainfall is about 2500mm and humidity 69% (Peeyus kutty, 2008; Kerala forest and wildlife department, 2013). PTR is covered mostly with evergreen, semi-evergreen, moist deciduous forests, and grasslands make up the rest of the area. Sporadic patches of 'sholas', which are sub tropical montane forests, occupy the crest and crevices of high altitude tracts (Peeyus kutty, 2008; Kerala forest and wildlife department, 2013). Elephant (*Elephas maximus* Linnaeus, 1758), Gaur (*Bos gaurus* Hamilton Smith, 1827), Sambar deer (*Cervus unicolor* Kerr, 1792), Barking deer [*Muntiacus muntjak* (Zimmermann, 1780)], Nilgiri langur [*Trachypithecus johnii* (Fischer, 1829)] and Bonnet macaque [*Macaca Radiata* (Geoffroy, 1812)] are the major mammals present in PTR (Kerala forest and wildlife department, 2013).

Sampling: Dung beetles were sampled using dung baited pitfall traps on a seasonal basis during 2009-2011 period from low elevation shola (1200 msl), evergreen (1000 msl) and deciduous (650 msl) forests in the PTR. Ten pitfall traps made of plastic basin (10 cm diameter, 15 cm deep), spaced at 50m interval between traps were placed to minimize trap interference (Larsen and Forsyth, 2005). Trap contents were collected at 12 h interval (6:00-18:00h and 18:00-6:00h) to separate diurnal and nocturnal species. Collected beetles were identified to species level using Arrow (1931) and Balthasar (1963) by the authors and were confirmed by comparing with the verified specimens in the collections of St. Joseph's College, Devagiri, Calicut. Identified specimens will be deposited in the museum of Zoological Survey of India, Western Ghats regional centre, Calicut. Species were sorted into three functional guilds namely, dwellers (endocoprids), rollers (telecoprids) and tunnelers (paracoprids) following Cambefort and Hanski (1991) and three temporal guilds (noctural/diurnal/generalists) following Krell *et al.* (2003). To assess the value of particular species as indicators of habitat change, the indicator species value (ISV) using the Indicator Value Method (IndVal) (Dufreˆne and Legendre, 1997) was calculated. Species with *IndVal* values, greater than 70% were regarded as characteristic indicator species and those between 50% and 70% were considered as detector species (McGeoch *et al*., 2002). Species diversity was calculated using Margalef's richness and Shannon diversity indices. Species compositions among habitats were compared with Bray-Curtis similarity coefficient. Significance of variation in the overall abundance among forest types were tested with Kruskal –Wallis test followed by Mann –Whitney test, diversity and species richness with one-way ANOVA followed by Tukey's test and guild

composition with Chi square test. Diversity analyses were done with PRIMER 5 software version 5.2.9 (Clarke and Gorley, 2001) and statistical analyses were done with MINITAB software (Minitab, 2010).

RESULTS

Thirty species belonging to 9 genera and five tribes were collected with 19 speceis from shola forests, 30 from evergreen forests and 20 from deciduous forests (Table 1). *Onthophagus ensifer* and *Caccobius meridionalis* were the dominant species in the shola and evergreen forests and *O. ensifer* and *O. fasciatus* in the deciduous forests. Overall abundance (H=12.50, DF=2, $P<0.05$) and species richness (F=5.08, DF=2, $P<0.05$) were higher in the evergreen forests. Diversity did not vary between the three forest vegetation types (F=2.01, DF=2, $P>0.05$). Highest similarity was between the dung beetle assemblages of the evergreen and shola forests (59.41%). Eight species namely, *Copris repertus, Onthophagus amphinasus, O. manipurensis, O. bronzeus, O. deflexicollis, O. bifasciatus, O. cavia* and *O. tritinctus* were specific to the evergreen forests and four species namely, *Onthophagus dama, O. duporti, O. favrei* and *O. refulgens* to deciduous forests. Nine generalist species namely, *Caccobius meridionalis, Paracopris cribratus, P. davisoni, P.signatus, Tibiodrepanus setosus, Onthophagus ensifer, O.fasciatus, O.kchatriya* and *O.rectecornutus* were present in all the three forests.

Beetles belonging to tunneler, roller and dweller functional guilds were recorded from the shola and evergreen forests whereas rollers were not recorded from the deciduous forests (Table 1). Functional guild composition based on abundance varied among the three forests (χ^2=21.08, DF=4, $P<0.05$). Tunnelers were the dominant guild (shola:98.01%, evergreen:95.42%, deciduous:99.10%) with low abundance of dwellers and rollers in all the three forest types. Temporal guild composition varied in abundance (χ^2=102.45, DF= 4, $P<0.05$) with diurnal guild dominating the assemblage in all forests types (shola: 87.83%, evergreen: 61.54%, deciduous: 74.35%). No vegetation specific indicator species were recorded. Based on the *Indval* scores, *Caccobius meridionalis*, *Onthophagus ensifer* and *Onthophagus fasciatus* were the detector species in the shola forests, *Paracopris cribratus* in the evergreen forests and *Onthophagus ensifer* and *Onthophagus fasciatus* in the deciduous forests (Table 1; Fig. 1).

DISCUSSION

Comparatively high abundance, species richness and species specificity of dung beetles in the evergreen forests than in the other forest types indicates that vegetation differences directly affect dung beetle populations in PTR. As evergreen forests are the major component of the vegetation in PTR, the resulting large area effect (Nichols *et al.,* 2007) and abundant dung resource availability in the evergreen forests (Kerala Forest and Wildlife Department, 2013) could be the reasons for high species richness in the evergreen forests compared to other forest types. High similarity in species composition between evergreen and shola forests

Table 1. Abundance, guild composition and *Indval* values of dung beetle assemblages in the three forest vegetation types at Periyar Tiger Reserve during 2009-11 period [*T=tunneler, D=dweller, R=roller; N=nocturnal, D=diurnal, G=generalist*].

	Species	Functional guild	Temporal guild	Shola		Evergreen		Deciduous	
				Mean ± SD	*Ind val*	*Mean* ± SD	*Ind val*	*Mean* ± SD	*Ind val*
1	*Onthophagus ensifer*	T	D	6.57 ± 9.13	56.6	3.67± 5.32	46.6	5.17 ± 6.78	63.3
2	*Caccobius meridionalis*	T	D	4.13 ± 5.79	56.6	6.13 ± 14.27	30.0	0.40 ± 1.00	23.3
3	*Onthophagus fasciatus*	T	D	2.10 ± 3.42	50.0	0.83± 1.46	33.3	4.87 ± 7.87	60.0
4	*Onthophagus rectecornutus*	T	N	0.47 ± 1.50	20.0	0.13± 0.35	13.3	1.53 ± 2.46	43.3
5	*Onthophagus furcillifer*	T	D	0.40 ± 0.93	23.3	0.37± 0.96	16.6	0.00 ± 0.00	0
6	*Onthophagus kchatriya*	T	G	0.30 ± 0.92	13.3	0.13± 0.43	10.0	0.07 ± 0.25	6.6
7	*Onthophagus andrewesi*	T	G	0.27 ± 0.64	16.6	0.07 ± 0.25	6.6	0.00 ± 0.00	0
8	*Tibiodrepanus setosus*	D	G	0.20 ± 0.55	13.3	0.07 ± 0.37	3.3	0.03 ± 0.18	3.3
9	*Onthophagus amphicoma*	T	G	0.17 ± 0.38	16.6	0.00 ± 0.00	0	0.13 ± 0.43	10.0
10	*Onthophagus vividus*	T	G	0.10 ± 0.55	3.3	0.00 ± 0.00	0	0.03 ± 0.18	3.3
11	*Catharsius molossus*	T	G	0.07 ± 0.25	6.6	0.13 ±0.57	6.6	0.00 ± 0.00	0
12	*Onthophagus castetsi*	T	G	0.07 ± 0.25	6.6	1.73 ± 2.55	46.6	0.00 ± 0.00	0
13	*Paracopris cribratus*	T	N	0.03 ± 0.18	3.3	1.83 ± 3.30	56.6	0.07 ± 0.37	3.3
14	*Paracopris davisoni*	T	G	0.03 ± 0.18	3.3	0.03 ±0.18	3.3	0.03 ± 0.18	3.3
15	*Paracopris signatus*	T	N	0.03 ±0.18	3.3	0.07 ±0.25	6.6	0.20 ± 0.61	13.3
16	*Ochicanthon nitidus*	R	G	0.03 ± 0.18	3.3	0.03 ± 0.18	3.3	0.00 ± 0.00	0
17	*Onthophagus vladimiri*	T	G	0.03 ± 0.18	3.3	0.10± 0.40	6.6	0.00 ± 0.00	0
18	*Sisyphus longipes*	R	G	0.03 ± 0.18	3.3	0.37± 0.81	20.0	0.00 ± 0.00	0
19	*Sisyphus neglectus*	R	D	0.03 ± 0.18	3.3	0.20±0.55	13.3	0.00 ± 0.00	0
20	*Copris repertus*	T	G	0.00 ± 0.00	0	0.07 ±0.25	6.6	0.00 ± 0.00	0
21	*Liatongus indicus*	D	G	0.00 ± 0.00	0	0.17 ± 0.59	10.0	0.10 ± 0.40	6.6

	Species	Functional guild	Temporal guild	Shola		Evergreen		Deciduous	
				Mean ± SD	*Ind val*	*Mean* ± SD	*Ind val*	*Mean* ± SD	*Ind val*
22	*Onthophagus amphinasus*	T	G	0.00 ± 0.00	0	0.63 ± 2.30	10.0	0.00 ± 0.00	0
23	*Onthophagus bifasciatus*	T	G	0.00 ± 0.00	0	0.03 ± 0.18	3.3	0.00 ± 0.00	0
24	*Onthophagus bronzeus*	T	G	0.00 ± 0.00	0	0.13 ± 0.43	10.0	0.00 ± 0.00	0
25	*Onthophagus cavia*	T	G	0.00 ± 0.00	0	0.03 ± 0.18	3.3	0.00 ± 0.00	0
26	*Onthophagus cervus*	T	G	0.00 ± 0.00	0	0.13± 0.35	13.3	0.27 ± 0.74	13.3
27	*Onthophagus dama*	T	G	0.00 ± 0.00	0	0.10± 0.31	0	0.03 ± 0.18	3.3
28	*Onthophagus deflexicollis*	T	G	0.00 ± 0.00	0	0.00 ± 0.00	10.0	0.00 ± 0.00	0
29	*Onthophagus duporti*	T	G	0.00 ± 0.00	0	0.00 ± 0.00	0	0.07 ± 0.25	6.6
30	*Onthophagus favrei*	T	G	0.00 ± 0.00	0	0.00 ± 0.00	0	0.27 ± 0.69	13.3
31	*Onthophagus laevis*	T	G	0.00 ± 0.00	0	0.37± 0.72	26.6	0.03 ± 0.18	3.3
32	*Onthophagus manipurensis*	T	G	0.00 ± 0.00	0	0.23± 0.68	13.3	0.00 ± 0.00	0
33	*Onthophagus pacificus*	T	N	0.00 ± 0.00	0	0.20± 0.48	16.6	0.07 ± 0.37	3.3
34	*Onthophagus reflugens*	T	G	0.00 ± 0.00	0	0.00 ± 0.00	0	0.07 ± 0.37	3.3
35	*Onthophagus tritinctus*	T	G	0.00 ± 0.00	0	0.03± 0.18	3.3	0.00 ± 0.00	0
36	*Onthophagus turbatus*	T	G	0.00 ± 0.00	0	0.17± 0.59	10.0	0.60 ± 1.13	30.0

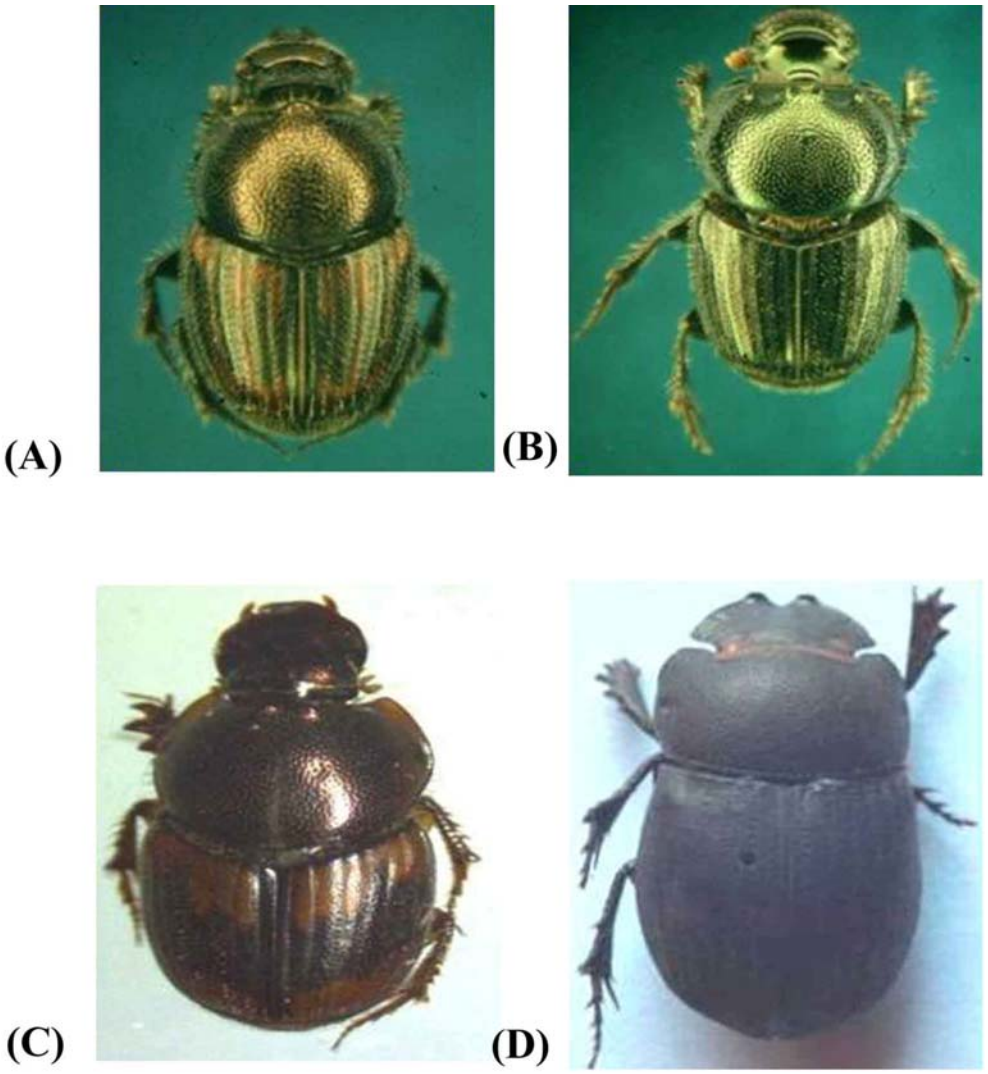

Figure 1. Detector species associated with the forests at Periyar Tiger Reserve: (A) *Caccobius meridionalis* - shola forest, (B) *Onthophagus ensifer* - shola & deciduous fore (C) *Onthophagus fasciatus* - shola & deciduous forests, (D) *Paracopris cribratus* - evergree forest.

is attributed to the movement of species between adjoining shola and evergreen forests and similarity in the habitat conditions in the two forests.

Low species richness in the low elevation shola forests at PTR agrees with the earlier reports from the high elevation shola forests in the Western Ghats (Sabu *et al.*, 2011). However, comparison between low and high elevation shola forests shows that species richness and abundance were higher and species composition was different in the low elevation shola forests at PTR compared to the high elevation shola forests of Eravikulam. *Onthophagus ensifer* and *Caccobius meridionalis* were the dominant species in the low elevation shola forests whereas *Onthophagus refulgens* and the wingless *Ochicanthon devagiriensis* dominated the high elevation shola forests (Sabu *et al.*, 2011). Difference in the species composition and dominance pattern in the low and high elevation shola forests is attributed to the altitudinal variations and the physiological adaptations of upper montane dung beetles to low temperature (Verdu *et al*., 2004).

Caccobius meridionalis and *Onthophagus ensifer* were the dominant species in the evergreen forests at PTR whereas *Onthophagus pacificus* and *O. furcillifer* were the dominant species at Nelliyampathy in the moist south Western Ghats (Latha, 2013). *Onthophagus ensifer* and *O. fasciatus* dominated the assemblage in the deciduous forests in PTR where as *Onthophagus andrewesi* and *Tibiodrepanus setosus* dominated the assemblage in the deciduous forests of Wayanad region in the north of moist south Western Ghats (Vinod, 2009) indicating both forest vegetation wise and region wise variation in the dominance of species in the moist south Western Ghats. Nine generalist species are common dung beetle species in the Western Ghats (Sabu, 2011) and their presence in all the three forests is indicative of their capacity to survive in all the three vegetation types.

Dominance of tunnelers, in all forest vegetation types in PTR and in other forests of the moist south Western Ghats indicate that dominance of tunnelers is typical of dung beetle assemblages in the moist south Western Ghats (Sabu *et al.*, 2006, 2007; Vinod and Sabu, 2007). Aggressive and superior competitive nature of tunnelers in utilizing the dung resources (Krell-Westerwalbesloh *et al*., 2004) might have contributed to their success and dominance in all the three forests. Low abundance of dwellers in all forest types is attributed to the low abundance of gaur in PTR (Veeramani, 2004) and the resulting lesser availability of undisturbed dung pats and the high abundance of tunnelers with superior competitive nature in PTR. Dominance of dwellers over rollers in the low elevation shola forests of PTR is in contrast to the non-record of the dweller guild in the high elevation shola forests of the Western Ghats (Sabu *et al*., 2011). Unlike the upper montane shola forests, shola forests at PTR are more frequented by elephants and steady availability of their dung pats could be the reason for the presence of dwellers.

Low abundance of rollers is attributed to low presence of dung pellet producing mammals such as deer and Nilgiri tahr and the limited availability of dung pellets preferred by rollers. Additionally, thick under storey vegetation in the forests in PTR (Kerala forest and Wildlife

Department, 2013) and the moist wet conditions in the forests that hinders ball rolling activities of roller dung beetles (Krell-Westerwalbesloh *et al*., 2004) could be the other factors leading to the low abundance of rollers in PTR. Occurrence of the rare primitive old world roller, *Ochicanthon*, a genus present in relict patches of moist forests in the Indo- pacific ecoregion (Latha *et al*., 2011) indicates that the forests of PTR has conditions favourable to support the group. Dominance of diurnal guild in the forests of PTR is arising from the preference of dung beetles for warm and dry conditions during day time and their higher activity during diurnal period (Gill, 1991) as well as peak in the defecation of mammals during day time (Davis *et al.*, 1997) and availability of fresh dung during day time.

Non-record of indicator species (habitat specialists) indicates that no species fulfilled the criteria of high specificity and fidelity in the three forest types and PTR is a habitat under stress (Anas *et al.*, 2013). Detector species are useful indicators of direction of change in a habitat than the highly specific indicator species restricted to a single state (Mc Geoch *et al*., 2002) and hence monitoring the detector species (*Caccobius meridionalis*, *Onthophagus ensifer* and *Onthophagus fasciatus* in the shola forests, *Paracopris cribratus* in the evergreen forests and *Onthophagus ensifer* and *Onthophagus fasciatus* in the deciduous forests) would enable in understanding the future direction of change in the various vegetation types in the PTR.

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Allelopathic interactions of certain *Musa* cultivars against *Odoiporus longicollis* (Olivier)

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ABSTRACT: *Odoiporus longicollis* is a major pest of the banana (*Musa)* cultivars that enormously feeds on the pseudostem and causes serious damage to banana cultivation. It is a monophagous pest of banana, which showed extreme preference to some commercially viable cultivars such as *Nendran*, (AAB) *Palayankodan,(AAB) Red banana (AAA)* and extreme non preference to some commercially non viable cultivars such as *Kadali, Kannan, Aattinkombu (*all *AA* types*)* and *Thenkaali (AAB).* Field study, diversity analysis and pest status of *Musa* cultivars in Chittar panchayt of Pathanamthitta district (Kerala) by Arc GIS software showed that maximum diversity of cultivars with minimum pest attack was seen in those wards which are ecotones with respect to forest and agro ecosystem. Rearing of *O.longicollis* larvae in *Thenkaali* and *Aattinkombu* has resulted mortality of them evidenced by hyperprotenemia and hyperuricemia of haemolymph. The HPTLC study has revealed that pseudostem of *Aattinkombu* and *Thenkaali* possessed three additional compounds which were not present in pest sensitive cultivars. The differential distribution of secondary metabolites in the pseudostem of the above two cultivars can also be felt by difference in the smell of freshly cut pseudostem.

KEYWORDS: *Odoiporus longicollis*, resistant *Musa* cultivars, ecotones, hyperproteineaemia, allelopathic ineractions

INTRODUCTION

Bananas (*Musa* spp) are the major food crop globally cultivated and consumed in more than 100 countries throughout the tropics and subtropics. They provide a staple food for millions of people (Tiwari *et al*., 2006). They are also providing a well balanced diet and also contribute to the livelihood through crop production, processing and marketing. In developing countries

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they are the most important food crop after rice, wheat and maize (INIBAP, 2000). They are the cheapest, plentiful and the most nourishing of all the fruits and are consumed by the rich and poor alike. The plants are considered as a symbol of prosperity and fertility due to its place as a token of goodwill in various religious practices and ceremonial functions (Agrawal *et al*., 2007).

Banana is attacked by a number of pests, that includes rhizome weevil *Cosmopolites sordidius* (Germer), banana aphid *Pentalonia nigronervosa* (Coq.), fruit and leaf scarring beetle *Nodostomata subcostatum* (Coq.) burrowing nematode *Radopholus similis*, among which major key pest is the banana pseudo stem borer, *Odoiporus longicollis* (Olivier), a monophagous pest of banana and plantains limiting the production and productivity, posing serious threats to the cultivation of bananas (Visalakshi *et al*.,1989). It was estimated that banana pseudo stem borer caused 10-90 percent yield loss depending on the growth stage of the crop and management efficiency (Padmanabhan and Sathiamoorthy, 2001).

Field study conducted in Chittar Panchayat of Pathanamthitta District has resulted in the identification of 21 cultivars of *Musa* .Genome classification and their pest status in relation to *O.longicollis* was carried out. Majority of the cultivars identified were triploid (either AAA or AAB), four are diploid with AA constitution and only one *Njalipoovan* with AB genetic constitution (Kavitha *et al*., 2015a) This pest exhibited extreme preference to commercially viable cultivars and extreme non preference to some commercially non viable and less common cultivars. Among the four cultivars which are resistant to pest, three (*Aattinkombu, Kannan* and *Kadali*) are diploid with AA type and only one *Thenkaali* is triploid with AAB type (Kavitha *et al*., 2015a) Farmers are reluctant to cultivate the commercially non viable and pest resistant cultivars because of some practical problems. All these aspects described in this paper.

MATERIALS AND METHODS

The study was carried out in Chittar Grama Panchayat, Pathanamthitta District. It is geographically located between 09Ú 18' - 09Ú20' of north longitude and 076Ú 53'- 076Ú 57' of east latitude. It is a high range area and elevation ranging from 59m to 334m above the sea level. The area enjoys a tropical climate with 2922 mm annual rainfall. Annual temperature range between 18 °C (64 °F) and 35 °C (95 °F) (The Statistics department, Pathanamthitta, 2004).The soil type was laterite. The South West region of the Panchayat falls into forest region and North East region consists of rubber plantation. The banana plots selected for the study falls in these two regions .The Panchayat consists of 13 wards, and five sites from each wards were selected based on accessibility and the agriculture records, available in the Panchayat office. A total of 65 sites were selected and latitude and longitude of sites were taken by using GPS (Geographical Positioning System).

Construction of map using ArcGIS Software

A total of 65 study sites from 13 wards were selected for the present study (5/ward). Local

farmers were consulted to identify the important banana growing areas within the Panchayat. Using Global Positioning System (GPS) during the field survey, the locations of each of these sites were collected. This information was used to construct maps using Arc GIS.

ArcGIS 9 (established in May 2004) is geographic information system (GIS) for working with maps and geographic information. It is used for creating and using maps; compiling geographic data; analyzing mapped information; sharing and discovering geographic information; using maps and geographic information in a range of applications; and managing geographic information in a database. The system provides an infrastructure for making maps and geographic information available throughout an organization, across a community, and openly on the Web. Today, GIS has evolved into a crucial tool for science based problem solving (Arc View GIS, 1996). The digitalized map of Chittar Panchayat was taken from the toposheet of Pathanamthitta prepared by Land Use Board, Vikas Bhavan (Toposheet No. 58 C /15/SE, Scale 1:25000, First Edition) .Fig 1Map showing land use Fig 2- Map showing study area were prepared from CED (Centre For Environment and Development, Thozhuvankodu, Trivandrum).

Study on the Diversity of *Musa* Cultivars

Various cultivars of *Musa* grown by the farmers in various fields were observed thoroughly for distinguishing features such as length and width of leaf, shape and colour of the pedicel, colour of the pseudostem, general appearance of the fruit bunch, peduncle of the fruit bunch, shape of the male flower buds, bract shape, and local name of the cultivar was recorded by interaction with the farmers.

Diversity Analysis

Diversity analysis was carried out by three standard methods such as Shannon Wiener diversity index, Simpson's diversity index, Species richness and Abundance (Southwood, 1978; Fager, 1972).

Experimental maintenance of larvae

The developing stages of banana stem weevil *O. longicollis* were collected from infected clones of common cultivars of *Musa* from the fields. Three month old *Musa* cultivar such as *Palayankodan*, a variety to which the pest showed strong preference to infestation, *Aattinkombu* and *Thenkaali*, two cultivars to which the pest showed no preference for infestation were taken for the study.

The crown of the plants were chopped down and a small depression was made at the cut end and third instar larvae (ten numbers) of *O. longicollis* were put into the depression made at the top of the live stump and observed for 4, 8 and 10 days. Number of live larvae after appropriate duration was observed for pupation, mortality and difference in their sizes from that of the control. The live larvae were collected and used for further studies.

The larvae were cleaned and placed on ice for immobilization. The hemolymph for biochemical studies was isolated by making a small cut on the neck of the larvae. Standard procedures were used for biochemical estimations- protein (Lowry *et al*., 1951), total free amino acid (TFAA) (Spies *et al*., 1957) uric acid (Standard Assay Kit) and SDS-PAGE (Laemmli, 1970). 10% acrylamide concentration was used for the separating gel and 4% for the stacking gel.

HPTLC analysis of the banana pseudostem: Banana pseudostem powder (2gm) was extracted in 95% ethanol for 8 hours. The extract was dried in vacuum rotary evaporator and the dried sample was dissolved in 2 ml of methanol.5 µl of solution was loaded on a pre coated (Silica gel, $60F_{254}$, 2mm thick) plate (Merk).The solvent mixture of chloroform: methanol (8.8:1.2). Anisaldihyde-Sulphuric acid mixture as location reagent .The plate with sample was heated as 105^0C for 5 minutes, Visualized and photographed. Data of quantitative estimation were analyzed statistically by ANOVA (Daniel, 2006).

RESULTS AND DISCUSSION

The land use map of Chittar pnchayat of Pathanamthitta District (Fig. 1) and the 65 study sites in that area is shown in Fig.2.All the 13 wards of this panchyat possessed commercial cultivation of *Musa* sp. The distribution and diversity of *Musa* cultivars in different wards of Chittar pnchayat is shown in Table 1.Three wards which shares boundery with Konni forest division of Kerala cited as 7,8 and 9 in this table showed rich diversity of *Musa* cultivars. The pest attack on cultivars were minimum in the above three wards. The pest status of these 21 cultivars were studied in field condition (Kavitha *et al* 2015a)and also by rearing the larvae in live stumps of different cultivars in experimental fields. The cultivars which caused 100% mortality of *O.longicollis* larvae on 10^{th} day of experimental maintenance was considered as pest resistant and they never showed any symptoms of pest attack under field condition (Kavitha *et al*.,2015b).Through field study we could prove that cultivars which are bearing large fruit bunches short duration to set flower, short duration to harvest with high market value were preferred by the farmers and they were *Nendran, Palayankodan (both AAB), Robusta ,Red banana(both AAA)* and *Njaalipoovan (AB)* and these cultivars were widely cultivated in the fields. The less common cultivars such as *Kadali, Aattinkombu, Kannan (all AA) and Thenkaali (AAB)* , were seen only within the premises of houses as lone clones and no commercial cultivation was seen in any of the study places. Interactions with farmers have proved that long duration for harvest, small size of the fruit bunch and low market value were the reasons which make the above cultivars as economically non viable. No symptoms of pest attack were seen on these pest resistant cultivars, such as *Kadali, Thenkaali, Aattinkombu* and *Kannan* (Fig.3).The above four cultivars took long duration to sprout once the suckers were separated from the mother and planted in a new site. Among the four resistant cultivars the *Kadali* cultivar is common because of its importance in temple worship (Shing, 2002).The plant and fruit bunch (*Kadali*) were very small and hence commercial cultivation was not common (Sunderbabu, 1983).

One of the very interesting features observed in the field study was that the cultivars such as

Fig 1.Land use map of Chittar Panchayat

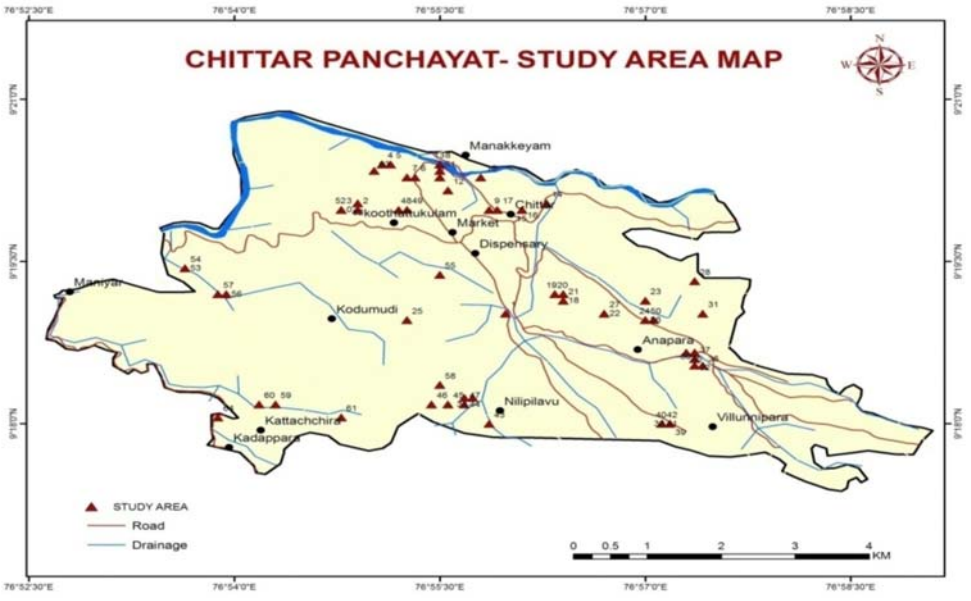

Fig 2.Study area map of Chittar Panchayat

Kannan

Aattinkombu

Thenkaali

Kadali

Fig3.Pest Resistant Cultivars

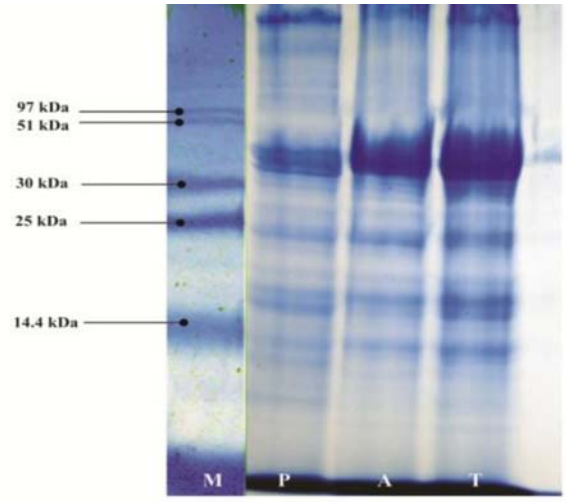

Fig 4. Electropherogram of hemolymph of *O. longicollis* larvae reared in resistant and susceptible Musa cultivars

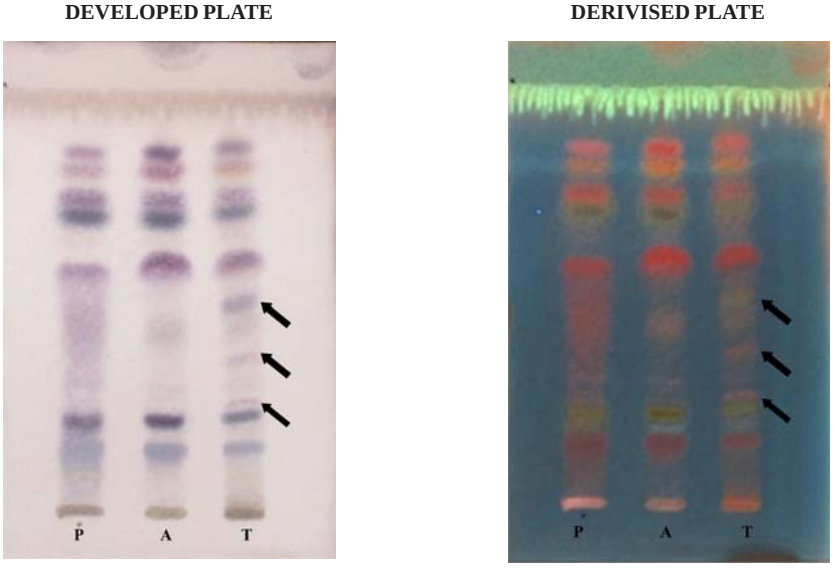

Fig 5.HPTLC Analysis Of Pseudostem of Three Musa Cultiars

P – *Palayankodan* A – *Aattinkombu* T – *Thenkaali*

Table 1.Comparison of *Musa* diversity in different wards of Chittar Panchayat

DIVERSITY STATISTICS				
WARD No.	**ABUNDANCE**	**SPECIES RICHNESS*1**	**SIMPSON INDEX*2**	**SHANNON WEINER INDEX*3**
1	22	12	0.18	2.22
2	20	12	0.14	2.95
3	42	10	0.25	1.61
4	37	11	0.26	1.92
5	52	9	0.32	1.44
6	34	10	0.21	2.16
7	18	19	0.09	4.27
8	20	15	0.10	4.00
9	15	17	0.08	4.62
10	43	13	0.24	1.70
11	50	6	0.32	1.40
12	22	15	0.14	4.23
13	21	12	0.10	2.21

*1 Number of different cultivars

*2 $D = \Sigma n(n-1) / N(N-1)$
(Values ranges from 0–1. **'0'** infinite diversity,**'1'** no diversity)

*3 $H' = \Sigma pi(ln\ pi)$
(Larger H = High diversity, values ranges from 0–5)

Red banana Palayankodan, and Nendran with very large pseudostem, leaves and fruit bunch are possessing high incidence of pest attack. On the other hand *Aattinkombu, Kadali* ,*Kannan* were very small with respect to their pseudostem, fruit bunch and leaves. The literature survey very well agreed with our observation and showed that *Nendran Red banana* and *Palayankodan* are triploid (Wang, 2010).It was well known that plants which are triploid or polyploids possessed high vegetative growth and at the same time the secondary metabolites present in them are very low. This may be the reasons for high incidence of pest attack in such cultivars *(*Wyiniger, 1962) and there are previous reports that. *O.longicollis* possessed extreme host specificity (Karr, 1983).

TABLE 2.Total Protein, Total Free Amino Acid (TFAA) And Uric Acid Levels in the Hemolymph of *Odoiporus longicollis* Maintained In Three *Musa* cultivars

Cultivars in which larvae were maintained*	LARVAL HEMOLYMPH		
	Total Protein (µg/ml)	Total Free Amino Acid (µg/ml)	Uric Acid (µg/100 ml)
Palayankodan	320.11 ± 6.52	498.07 ± 7.82	7.89 ± 0.68
Attinkombu	460.06 ± 7.39	587 ± 8.61	9.801 ± 0.91
Thenkali	500.1 ± 8.26	383 ± 6.76	10.99 ± 0.99

Larvae were maintained in four days in the pseudostem
Values are Mean ± S.D. All values are significant at 0.05 levels on compiring with control (*Palayankodan*) n=6

O.longicollis larvae maintained in *Palayankodan* has successfully pupated, but the larvae maintained in *Thenkaali* and *Aattinkombu* showed significant changes from control such as weakness and absence of wriggling movements. When they were carefully dissected out from the pseudostem on the fourth day and kept on a fresh pseudostem they were unable to bore into it indicating extreme weakness. Hyperproteinaemia and hyperururicemia together with increase in Total free amino acids (TFAA) were observed in larvae maintained *Aattinkombu* for four days (Table.2). Hyperproteinaemia and hyperururicemia together with decrease in TFAA were observed in larvae maintained *Thenkaali* for four days (Table.2). Increased amount of uric acid in intoxicated larvae of both cultivars may due to increased catabolism of amino acids or nucleotides. More than 85% of larvae were dead on the eighth day of maintenance and 100% mortality of larvae was seen on the 10 th day of maintenance in *Thenkaali* and *Aattinkombu*.

Hyperproteinaemia of hemolymph can be considered as generalized stress response of insect larvae, which were subjected to various types of stress. Hyperproteinaemia was observed in other Coleopteran larvae such as *Oryctes rhinoceros* in the presence of various stress conditions such as infection of *Bacillus thuringiensis*, exposure to cold shock, and infection by ectoparasitic mite *poecilochirus* sp., and antigen challenge (Adhira *et al*., 2010, Adhira, 2015). It is known that uric acid is produced principally in the cells of the fat body and is released into the hemolymph, which is then transported to the malphigian tubules to be excreted out. Our findings are agreeing with the observations of investigators on *Spodoptera litura* under various stress condition (Tripathy and Singh, 2002).

SDS-PAGE of hemolymph of *O.longicollis* larvae maintained in *Thenkaali* and *Aattinkombu* were proved that there was elevation of protein content in quantitative estimations and also evidenced by increase in the thickness of many protein bands. Many small polypeptides are

vanished or lost during the course of toxicity, complete disappearance of a series of low molecular weight protein were observed in larvae maintained in non-preferred varieties which was attested through GEL-DOC analysis of the electrophorogram (Fig.4). In GEL-DOC analysis it is clear that larvae in *Thenkaali* cultivar showed protein band with molecular weight 16.97, 29.78,41.64 and 226.03 are not seen in *Palayankodan*.Similarly larvae maintained in *Aattinkombu* showde protein band with molecular weight 21.96, 27.58, 49.06 and 124.56 are not found in *Palayankodan*.

The High Performance Thin Layer Chromatography (HPLC) study has revealed that pseudostem of *Palayankodan*, *Aattinkombu* and *Thenkaali* has 9-10 compounds which are common in three varieties which gave positive reaction with colouring reagent, Anisaldehyde-Sulphuric acid mixture. *Thenkaali*, a pest resistant cultivars possessed three additional compounds which gave positive reaction to the above colouring reagent, but *Aattinkombu* exhibited compounds almost identical to *Palayankodan* and at the same time, some compounds in excess quantities than *Palayankodan* (Fig.5). It was reported by other investigators that the pest had least affinity towards certain *Musa* cultivars (Tiwari, 1971). The differential distribution of secondary metabolites in the pseudostem of the above two cultivars can also be felt by difference in the host plant odour. It may be the reason for selective preference of the pest. The non-preference of *O. longicollis* towards *Thenkaali, Aattinkombu* may be the presence of certain volatile secondary metabolites. The chromatogram of the pseudo stem of the three varieties such as *Palayankodan, Thenkaaali and Aattinkombu* has revealed that observed non-preference *by O. longicollis* may be due to the presence of additional compounds in their pseudo stem. The odour differences of the sap of these three cultivars were noticed. The mother weevil *(O.longicollis*) with sensitive antennae could differentiate the most favorable host plant for their next generation and this may be the reason for no symptoms of pest attack on *Thenkaali and Aattinkombu*.

In India (Isahaque, 1978) showed that the banana cultivars such as 'Bhimkal',Kaskal and Jhajee were completely free of infestation by *O. longicollis*. Resistance in these three varieties appeared to be connected with their broad, thick and compact leaf sheaths and pseudostems, although chemical antibiosis may also have been a contributory factor. Vevai (1971) has repoted that some cultivars with excess phenolic compounds showed no pest attack in field condition.

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Revision of the whitefly genus *Martiniella* Jesudasan and David (Hemiptera: Aleyrodidae) with a new record of *Martiniella sepangensis* (Martin and Mound) from India

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ABSTRACT: The whitefly genus *Martiniella* Jesudasan and David is reviewed and the generic characters have been redefined. Five species of *Aleuroclava* viz., *A. baccaureae* (Corbett), *A. fici* (Corbett), *A. macarangae* (Corbett), *A. sepangensis* Martin and Mound and *A. srilankaensis* (David) have been assigned to *Martiniella* proposing new combinations. *M. sepangensis* (Martin and Mound) so far known from Malaysia is reported for the first time from India and the species is redescribed. Key to the species of the genus *Martiniella* is given.

KEYWORDS: Indian Aleyrodidae, *Martiniella sepangensis*

INTRODUCTION

Jesudasan and David (1990) proposed the whitefly genus *Martiniella* for two species of *Aleurotuberculatus* viz., *A. canangae* and *A. macarange* described by Corbett (1935), with the former being the type species. Martin (1999) synonymised *Martiniella* with *Aleuroclava* observing as follows "Jesudasan and David (1990) proposed the genus *Martiniella* for two species described by Corbett (1935), *Aleurotuberculatus canangae* and *A. macarangae*, using the presence of very much enlarged, jointed, cephalic and first abdominal setae as the diagnostic separation from *Aleuroclava*, although unusual setae of this type are sometimes present in species of *Taiwanaleyrodes* and *Dialeurodes*, and this character has also been seen to vary within samples (personal observations), *Martiniella* was therefore, considered as a junior synonym of *Aleuroclava*". In this connection Sundararaj and Dubey (2004) emphasized that the presence of very much enlarged, jointed, cephalic and first abdominal

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setae form a distinct diagnostic character in separating *Martiniella* from all known species of *Aleuroclava*. Thus placement of all the described species of *Martiniella* under *Aleuroclava* by Martin and Mound (2007) is not justifiable. In view of this Sundararaj and Pushpa (2011) reinstated the generic status of *Martiniella*. Further, no variations were observed within samples with regard to the jointed nature of setae in the species of *Martiniella* collected by different workers from 1976 onwards. A critical evaluation of the jointed nature of setae revealed that the base of the seta is nothing but an elongated extension of the cuticle in the form of elongate tubercle bearing the seta at its apex. Hence, the generic characters have been redefined and based on the original description five species of *Aleuroclava viz., A. baccaureae* (Corbett), *A. fici* (Corbett), *M. macarangae* (Corbett), *A. sepangensis* Martin and Mound and *A. srilankaensis* (David) have been assigned to *Martiniella* proposing new combinations. Further *M. sepangensis* (Martin and Mound) so far known from Malaysia is reported for the first time on *Macaranga* sp. from India and a redesscription of the species is given.

MATERIALS AND METHODS

The present study was based on the whitefly materials collected from various localities of south India during the period 2005-15 as well as the type specimens and other specimens of *Martiniella* available at the collections of Institute of Wood Science and Technology (IWST). The whitefly infested leaves were collected from the host plants and permanent mounts of the puparia were prepared by adopting the method suggested by David and Subramaniam (1976). The best mounts were obtained from puparia from which adults have emerged. Observations were made by using Nikon Optiphot T-2 EFD microscope and the identity of the whiteflies were confirmed.

RESULTS AND DISCUSSISON

Genus *Martiniella* Jesudasan and David, 1990

Type species: *Aleurotuberculatus canangae* Corbett, 1935. *J. Fed. Malay. St. Mus.* **17**: 827–828; by original designation.

Martiniella canangae (Corbett) Jesudasan and David, 1990. *FIPPAT Entomology Series,* **2**: 1-13.

Aleuroclava canangae (Corbett) Martin, 1999. *CSIRO Entomology Technical Paper,* **38**: 197 pp.

Martiniella canangae (Corbett) Sundararaj and Dubey, 2004. *Entomon*, **29** (4): 357-360.

Aleuroclava canangae (Corbett) Martin and Mound, 2007. *Zootaxa*, **1492**: 10.

Martiniella canangae (Corbett) Sundararaj and Pushpa, 2011: 509. In: *Advancements in Invertebrate Taxonomy and Biodiversity.* Gupta, Rajiv K. (Ed.), Agrobios (International), 552 Pp.

Diagnosis. Puparia small, ≤ 0.66 mm long and white with dorsal tubercles and granules; margin finely crenulate, caudal tracheal pore distinct while thoracic tracheal pore regions either distinct or slightly differentiated from margin; submargin often separated from the dorsal disc by a thin submarginal fold; thoracic and caudal tracheal folds distinct; cephalic and first abdominal setae on elongated tubercles (tuberculate setae) which are generally mistaken as jointed setae. Vasiform orifice subcordate, notched at hind end; operculum filling the orifice, obscuring the lingula.

The puparia of *Martiniella* are easily distinguishable from *Aleuroclava* Singh by the smaller size and having cephalic and first abdominal setae on elevated long tubercles similar to the tuberculate setae of *Tuberaleyrodes* Takahashi and *Acanthaleyrodes* Takahashi. It also differs by presence of submarginal ventral fold from *Aleuroclava* though some of the species placed presently under *Aleuroclava* have submarginal ventral fold but they are not typical of the genus *Aleuroclava*. Further many included taxa which are initially placed under *Aleurotuberculatus* are not congeneric with the type species *A. complex* Singh.

The genus *Martiniella* differs from *Acanthaleyrodes* Takahashi in having only tuberculate cephalic and first abdominal setae and submarginal ventral fold and by not having vasiform orifice on an eminent elevated protuberance and by the absence of submarginal and subdorsal tubercualte setae. It also differs from *Tuberaleyrodes* Takahashi in having only the tuberculate cephalic and first abdominal setae and submarginal ventral fold and by the absence of submarginal and subdorsal tubercualte setae. Further it is observed beyond doubt that tuberculate nature of cephalic and first abdominal setae is not a variable character in the natural breeding populations of *M. indica* on *Michelia champaca*.

Key to puparia of the species of *Martiniella*

(Based on the puparial observation of Indian species and the original description of species reported from outside India)

1. Thoracic tracheal pores/clefts/folds indicated 2

-. Thoracic tracheal pores/clefts/folds not indicated. 6

2. Dorsal area not smooth, with papillae or tubercles 3

-. Dorsal area smooth, without papillae or tubercles . . . . . ***ayyari*** Sundararaj and David

3. Entire dorsum not smooth, with papillae, granules and tubercles; vasiform orifice cordate 4

-. Submedian area smooth, without papillae or granules, only subdorsum with papillae and granules; vasiform orifice subrectangular . . . . . ***lefroyi*** Sundararaj and David

4. Submedian area of cephalothorax without three pairs of enlarged tubercles; subdorsum only with microtubercles. 5

-. Submedian area of cephalothorax with three pairs of enlarged tubercles; entire dorsum with microtubercles. . . . . ***canangae*** (Corbett)

5. Abdominal segment sutures with thick corrugations; .....***macarangae*** (Corbett)

-. Abdominal segment sutures without corrugations; three rows of small pores absent in the abdomen; microtubercles along the segment sutures absent; inner subdorsum without microtubercles, outer subdorsum along the submargin with microtubercles . . . . . ***papillata*** Sundararaj and Dubey

6. Submargin without large subcircular lobes 7

-. Submargin with three pairs of large sub circular lobes.. . . . . ***tripori*** (Dubey and Sundararaj)

7. Abdominal segments with median tubercles. 8

-. Abdominal segments without median tubercles 10

8. Median tubercles on abdominal segments not extending along the segment sutures; subdorsum without microtubercles 9

-. Median tubercles on abdominal segments extending along the segment sutures; subdorsum with microtubercles. . . . . ***fletcheri*** (Sundararaj and David)

9. Abdominal segments II to IV with median tubercles; caudal furrow not closed at its anterior end . . . . . ***srilankaensis*** (David)

-. Abdominal segments II-V and VII with chitinised thickenings and extending into subdorsal area; caudal furrow closed at its anterior end . . . . . ***sepangensis*** (Martin and Mound)

10. Basal tuberculate and apical setae of the cephalic and I abdominal setae are not equal in length. 11

-. Basal tuberculate and apical setae of the cephalic and I abdominal setae are about equal in length . . . . . ***indica*** (Singh)

11 Apical setae of the tubeculate cephalic and I abdominal setae are about twice as long as the basal tuberculate. . . . . ***fici*** (Corbett)

-. Basal tuberculate of the tubeculate cephalic and I abdominal setae are very long, more than double the length of the apical setae. . . . . ***baccaureae*** (Corbett)

Species of the genus *Martiniella*

1. ***Martiniella ayyari*** Sundararaj and David

Martiniella ayyari Sundararaj and David, 1993. *Entomon* **18** (1&2): 95-98.

Aleuroclava ayyari (Sundararaj and David) Martin, 1999. *CSIRO Entomology Technical Paper,* **38**: 31.

Martiniella ayyari: Sundararaj and Dubey, 2004. *Entomon*, **29** (4): 357-360.

Aleuroclava ayyari (Sundararaj and David) Martin and Mound, 2007. *Zootaxa*, **1492**: 9.

Martiniella ayyari: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity*: 510

Material examined: India: Tamil Nadu, paratype puparium, on *Mussaenda* sp., 4.viii.1987, R.Sundararaj.

Hosts: *Mussaenda* sp. (Sundararaj and David, 1993): *Litsea ghatica* (Meganathan and David, 1994).

Distribution: India: Tamil Nadu (Sundararaj and David, 1993).

2. ***Martiniella baccaureae*** (Corbett) **Comb. nov.**

Taiwanaleyrodes baccaureae Corbett, 1935: 839.

Aleuroclava baccaureae: Manzari and Quicke, 2006: 2470.

Material examined: None.

Host: *Baccaurea motleyana*. (Corbett, 1935).

Distribution: Malaya: Pudu (Corbett, 1935).

3. ***Martiniella canangae*** (Corbett) **Stat. Rev.**

Aleurotuberculatus canangae Corbett, 1935: 827.

Martiniella canangae (Corbett) Jesudasan and David, 1990: 1-13.

Aleuroclava canangae: Martin, 1999: 31.

Martiniella canangae: Sundararaj and Dubey, 2004: 357 - 360.

Material examined: None.

Host: *Cananga odorata*, *Psidium guajava* (Corbett, 1935).

Distribution: Malaya: Kuala Lumpur (Corbett, 1935).

4. ***Martiniella fici*** (Corbett) **Comb. nov.**

Taiwanaleyrodes fici Corbett, 1935: 838.

Aleuroclava fici: Manzari and Quicke, 2006: 2470.

Material examined: None.

Host: *Ficus* sp., *Euphorbia pulcherrima* (Corbett, 1935).

Distribution: Malaya: Kuala Lumpur (Corbett, 1935).

5. ***Martiniella fletcheri*** (Sundararaj and David) (Fig. 1-3)

Taiwanaleyrodes fletcheri Sundararaj and David, 1992. **29** (4):15- 20.

Aleuroclava fletcheri: Manzari and Quicke, 2006. *J. Nat. Hist.*, **40**, 2470.

Martiniella fletcheri: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity*: 510

Material examined: India: Karnataka: Sakleshpura, 4 puparia, on *Helicteres isora*, 15.x.05, R. Pushpa; Yellapur, 6 puparia, on *Michelia champaca*, 14.xii.05, R. Sundararaj; Kerala: Pandalam, 11 puparia, on *Macaranga peltata*, 27.iii.07, R. Pushpa; Singampara (Palakkad), 2 puparia, on *Dillenia pentagyna*, 22.x.06, R. Sundararaj; Palakkad, 2 puparia, on *Mallotus philippensis*, 23.x.06, R. Sundararaj; Palode, 13 puparia, on *Macaranga peltata*, 25.iii.07, R. Pushpa; Tamil Nadu: Kuppam, 1 puparium, on *Ficus hispida*, 23.xi.07, R. Pushpa; Courtalam, 4 puparia, on *Mallotus philippensis*, 22.iii.07, R. Pushpa; Unnamalaikadai, 10 puparia, on *Croton malabaricus*, 16.x.06, P. Philomena; Unnamalaikadai, 4 puparia on *Tectona grandis*, 11.xi.06, R. Pushpa.

Hosts: *Mallotus* sp., *Tectona grandis, Hemidesmus indicus* (Sundararaj and David, 1992), *Lannea coromandelica, Litsea bourdilloni* (Dubey and Ko, 2008). *Croton malabathrum. Dillenia pentagyna, Helicteres isora, Macaranga peltata*, *Mallotus*

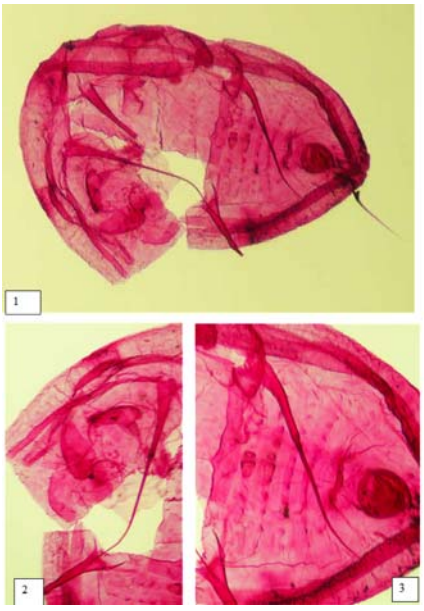

Fig. 1-3: *Martiniella fletcheri* (Sundararaj and David): 1. Puparium; 2. Cephalothorax with tuberculate cephalic setae; 3. Abdomen with tuberculate first abdominal setae

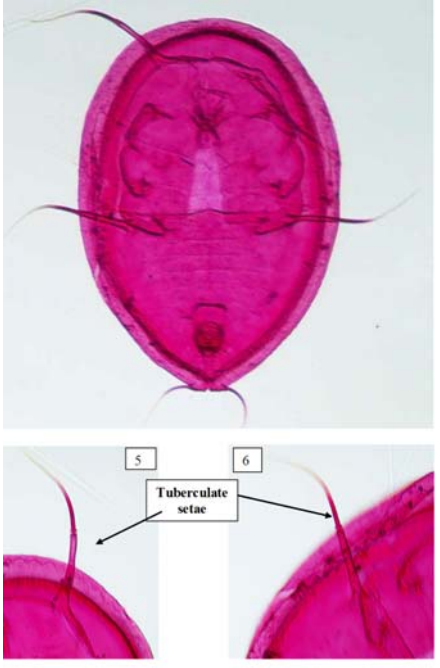

Fig. 4-6: *Martiniella indica* (Singh): 4. Puparium; 5. Tuberculate cephalic setae; 6. Tuberculate first abdominal setae

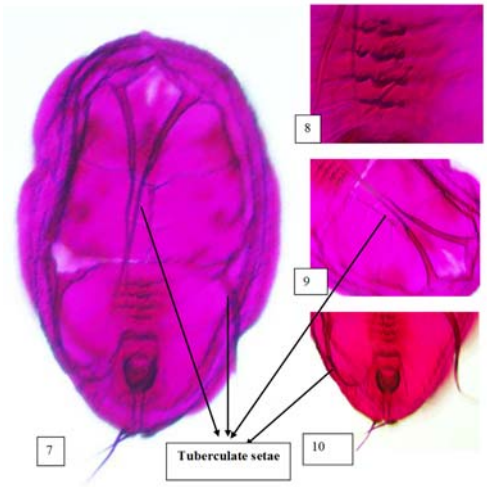

Fig. 7-10: ***Martiniella sepangensis*** **(Martin and Mound): 7. Puparium; 8. Abdominal segments with corrugated sutures; 9. Tuberculate cephalic setae; 10. Tuberculate first abdominal setae with vasiform orifice**

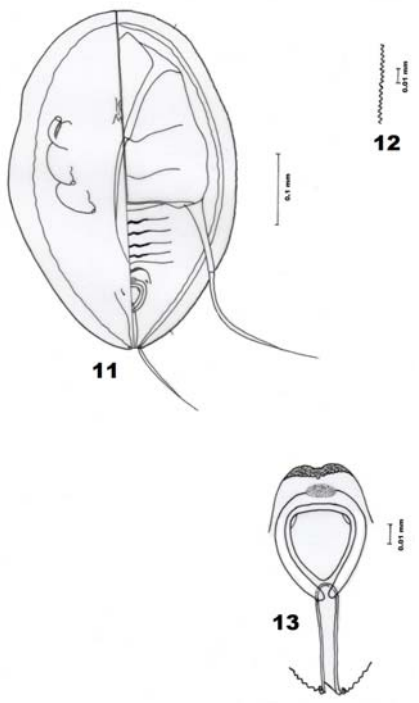

Fig. 11-13: Line diagram, ***Martiniella sepangensis*** **(Martin and Mound): 11. Puparium; 12. Margin; 13. Vasiform orifice**

philippensis, *Michelia champaca* (Sundararaj and Pushpa, 2011).

Distribution: India: Tamil Nadu, Kerala (Sundararaj and David, 1992); Karnataka: (new distribution record).

6. ***Martiniella indica*** (Singh) (Fig. 4-6)

Aleurothrixus indica Singh, 1931. *Mem. Dept. Agric. India, Ent. Ser.*, **12** (1): 84-85.

Taiwanaleyrodes indica (Singh) Takahashi, 1935. *Rec. Dept. Agric. Govt. Res. Inst. Formosa,* **66**: 55; David and Subramaniam, 1976. *Rec. Zool. Surv. India,* **70**: 212-213.

Aleuroclava indica: Manzari and Quicke, 2006. *J. Nat. Hist.*, **40**, 2470.

Martiniella indica: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity:* 510

Material examined: India: Andhra Pradesh: Rajendra Nagar (Hyderabad), 13 puparia, on *Ficus* sp., 5.iii.07, R. Sundararaj; Karnataka: Bangalore, 11 puparia, on *Michelia champaca*, 17.v.07, R. Pushpa; Moodabidri, 11 puparia, on *Ficus hispida*, 14.x.05, R. Pushpa; Sakleshpura, 6 puparia, on, *Ficus exasperata*, 15.x.05, R. Pushpa; IWST Campus (Bangalore), 12 puparia, on *Michelia champaca*, 22.v.2012, T. Amuthavalli; Yelahanga (Bangalore), 4 puparia, on *Michelia champaca*, 21.ii.2014, R. Sundararaj; IWST Campus (Bangalore), 3 puparia, on *Holigarna arnottiana*, 8.xii.2014, R. Sundararaj; IWST Campus (Bangalore), 21 puparia, on *Michelia champaca*, 7.xii.2014, R. Sundararaj; Varthahalli, 7 puparia, on *Ficus hispida*, 2.ii.2015, D. Vimala; Kerala: Pullanikadu (Thrissur), 10 puparia, on *Litsea* sp., 24.x.06, R. Sundararaj.

Hosts: *Michelia champaca* (Singh, 1931); *Dillenia indica* (Corbett, 1935); *Machilus* sp. (Takahashi, 1935); *Celtis tetrandra, Elaeocarpus tuberculatus, Ficus carica, Helietres isora, Litsea bourdillonii, L. ghatica, Scolopia crenata* (Meganathan & David, 1994); *Ficus* sp., *Ficus exasperata*, *Ficus hispida, Litsea* sp. (Sundararaj & Pushpa, 2011); *Castanopsis indica* (Lalneihpuia and William, 2011); *Alseodaphne semicarpifolia, Palaquium ellipticum* (Dubey and David, 2012); *Holigarna arnottiana* (new host record).

Distribution: India (Singh, 1931); Hong Kong, Taiwan and Malaya (Takahashi, 1941).

7. ***Martiniella lefroyi*** Sundararaj and David

Martiniella lefroyi Sundararaj and David, 1993. *Entomon* **18** (1&2): 97-99.

Aleuroclava lefroyi: Martin, 1999. *CSIRO Entomology Technical Paper,* **38**: 31.

Martiniella lefroyi: Sundararaj and Dubey, 2004. *Entomon*, **29** (4): 357-360.

Martiniella lefroyi: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity:* 511

Material examined: India: Maharashtra: Mahableshwar, paratype puparium on *Elatostemma* sp., 28.iii.1987, B. V. David.

Host: *Elatostemma* sp. (Sundararaj and David, 1993).

Distribution: India: Maharashtra (Sundararaj and David, 1993).

8. ***Martiniella macarangae*** (Corbett) **Stat. Rev.**

Aleurotuberculatus macarangae Corbett, 1935. *J. fed. Malay. St. Mus.,* **17**: 829.

Martiniella macarangae (Corbett) Jesudasan and David, 1990. *FIPPAT Entomology Series,* **2**: 1-13.

Aleuroclava macarangae (Corbett) Martin, 1999. *CSIRO Entomology Technical Paper,* **38**: 31.

Material examined. Nil.

Host. *Macaranga* sp. (Corbett, 1935).

Distribution: Malaya: Kuala Lumpur and Rawang (Selangor) (Corbett, 1935).

9. ***Martiniella papillata*** Sundararaj and Dubey

Martiniella papillata Sundararaj and Dubey, 2004. *Entomon*, **29** (4): 357-360.

Aleuroclava papillata: Martin and Mound, 2007. *Zootaxa*, **1492**: 11.

Martiniella papillata: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity:* 511

Material examined: India: Goa: Volpoi, paratype puparium, on *Xeromphis spinosa*, 27.ii.2001, A. K. Dubey.

Host: *Xeromphis spinosa* (Sundararaj and Dubey, 2004); *Buettneria aspera*, *Schima wallichii* (Lalneihpuia and William, 2011).

Distribution: India: Goa (Sundararaj and Dubey, 2004).

10. ***Martiniella sepangensis*** (Martin and Mound) **Comb. nov.** (Fig. 7 – 13)

Taiwanaleyrodes macarangae Corbett, 1935: 840.

Aleuroclava sepangensis: Martin and Mound, 2007: 11.

This species is reported for the first time from India. A detailed redescription of the species is given.

Puparium: Small, broadest across I abdominal segment; 0.45-0.50 mm long, 0.30-0.33 mm wide; oval, narrowing posteriorly; white, without secretion of wax; found singly on the under surface of leaves.

Margin: Finely crenulate, 40-42 crenulations in 0.1 mm. Anterior and posterior marginal setae respectively, 8 µm and 10 µm long. Thoracic tracheal pore regions not indicated while caudal tracheal pore distinct.

Dorsum: Submargin separated from the dorsal disc by a thin submarginal ventral fold, without striations; abdominal segments II-V and VII with chitinised thickenings and extending into subdorsal area, dorsum smooth without any granules or wavy markings. Longitudinal moulting suture reaching margin, transverse moulting suture reaching outer subdorsum.

Chaetotaxy: Two pairs of long tuberculate setae- cephalic setae 280-300 µm long (basal long elevated tubercle 105-108 µm long and the seta at apex 175 to 192 µm long) and first abdominal setae 225-240 µm long (basal long elevated tubercle 75-80 µm and the seta at apex 150 to 160 µm long); a pair of minute eighth abdominal setae cephalolaterad of vasiform orifice 5 µm long and a pair of submarginal caudal setae 115-125 µm long.

Vasiform orifice: Cordate, distinctly notched at caudal end with its lateral walls ridged, 42-47 µm long, 38-42 µm wide; operculum cordate, 22-25 µm long, 21-24 µm wide, filling the orifice and obscuring lingula, a transverse elliptical porous area at the anterior end of vasiform orifice. Thoracic tracheal furrows indistinct, caudal tracheal furrow distinct, cylindrical shape, closed at its anterior end, without any markings, 50-55 µm long, 11.5-12.5 µm wide. Pores and porettes not evident.

Venter: A pair of ventral abdominal setae 6 µm long, 19 µm apart. Antennae reaching base of prothoracic legs. Thoracic tracheal folds not indicated while caudal tracheal fold distinct.

Material examined: India: Karnataka: Varthahalli, 5 puparia on *Macaranga* sp., 3.ii.2015, D. Vimala.

Host: *Macaranga megalophylla* (Corbett, 1935); *Macaranga* sp.

Distribution: Malaya: Sepang (Selangor) (Corbett, 1935): India: Karnataka: Varthahalli (new distribution record).

11. ***Martiniella srilankaensis*** (David) **Comb. nov.**

Taiwanaleyrodes srilankaensis David, 1993: 29.

Aleuroclava srilankaensis: Manzari and Quicke, 2006: 2470.

Material examined: None.

Host: *Macaranga* sp. (David, 1993).

Distribution: Sri Lanka: Yattogoda (David, 1993).

12. ***Martiniella tripori*** (Dubey and Sundararaj)

Taiwanaleyrodes tripori Dubey and Sundararaj, 2006. *Entomon*, **31** (1): 73-76.

Aleuroclava tripori: Martin and Mound, 2007. *Zootaxa*, **1492**: 10.

Martiniella tripori: Sundararaj and Pushpa, 2011. *Advancements in Invertebrate Taxonomy and Biodiversity:* 511.

Material examined: India: Karnataka: Balehonnur, 9 puparia on *Ficus auriculata*, 5.vi.2013, T. Amuthavalli.

Host: Unidentified plant (Dubey and Sundararaj, 2006); *Ficus auriculata* (new host record).

Distribution: India: Kerala (Dubey and Sundararaj, 2006).

Martiniella is not considered as a valid genus with the assumption that the tuberculate nature of cephalic and first abdominal setae is a variable character without any scientific facts (Martin, 1999). In the present study it is observed beyond doubt that it is not a variable character and hence it is fit to consider *Martiniella* as a valid genus. It is a genus of Oriental region, so far reported from Hong Kong, India, Malaysia, Sri Lanka and Taiwan. It comprises 12 species, with the inclusion of those species originally placed in *Aleurotuberculatus* viz., *Martiniella canangae* (Corbett), *M. macarangae* (Corbett), and *Taiwanaleyrodes* viz., *M. baccaureae* (Corbett) **Comb. nov.,** *M. fici* (Corbett) **Comb. nov.,** *M. fletcheri* (Sundararaj and David), *M. indica* (Singh), *M. sepangensis* (Martin and Mound) **Comb. nov.** and *M. srilankaensis* (David) **Comb. nov.** With the six already known species viz., *M. ayyari* Sundararaj and David, *M. fletcheri* (Sundararaj and David), *M. indica* (Singh), *M. lefroyi* Sundararaj and

David, *M. papillata* Sundararaj and Dubey and *M. tripori* Dubey and Sundararaj and the record of *M. sepangenis* (Martin and Mound) from Karnataka brings the number of Indian species of *Martiniella* to seven. Six species viz., *M. baccaureae* (Corbett), *M. canangae* (Corbett), *M. fici* (Corbett), *M. indica* (Singh), *M. macarangae* (Corbett) and *M. sepangensis* (Martin and Mound) are reported from Malaysia and a species *M. srilankaensis* (David) from Sri Lanka. *M. indica* (Singh) is known to occur in India, Malaysia, Hong Kong and Taiwan and *M. sepangensis* (Martin and Mound) is now known from Malaysia and India

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Morphological characters and DNA barcodes to separate *Oenopia sauzeti* Mulsant and *O. mimica* Weise (Coleoptera: Coccinellidae), two externally similar lady beetles from the Indian subcontinent

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ABSTRACT: *Oenopia sauzeti* Mulsant and *O. mimica* Weise (Coleoptera: Coccinellidae) are externally very similar and commonly misidentified species of lady beetles distributed in the Indian subcontinent. Diagnostic characters including male genitalia are illustrated for these species to facilitate their identification. The *cox1 mtDNA* sequences of *O. sauzeti* and *O. mimica* (658 bp) had only 89% similarity upon pair-wise alignment, which distinguished them with 75 nucleotide differences, thus confirming that these are distinct species. DNA barcodes with accession numbers AGIMP042-15 for *O. sauzeti* and AGIMP043-15 for *O. mimica* were obtained.

KEY WORDS: *Oenopia*, Coccinellidae, DNA barcodes, morphology, Indian subcontinent

INTRODUCTION

Oenopia sauzeti Mulsant (1866) and *O. mimica* Weise (1902) are externally very similar sympatric species distributed in the Indian subcontinent (Mader, 1935; Miyatake, 1985; Poorani, 2002a, b). Of these two, *O. sauzeti* is fairly common and widely distributed throughout north, northwestern and northeastern India, Pakistan, Nepal, Bhutan, China and in parts of Southeast Asia. *Oenopia mimica* has a much more restricted distribution and is confined to the higher altitudes of Eastern Himalayas in India, Nepal, and Bhutan.

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Mader (1935) illustrated both species and described their diagnostic characters, particularly body size and elytral colour patterns. He observed that "*O. mimica* is as big as the smallest specimens of *O. sauzeti*". Iablokoff-Khnzorian (1979) synonymized *O. sauzeti* and *O. mimica*, perhaps misled by their external similarity. Miyatake (1985) restored them as valid species based on his studies on collections from Nepal Himalayas and summarized the morphological differences between the two species with illustrations. These two species were also illustrated and keyed in Poorani's (2002b) review of Indian species of *Oenopia*. Still, they continue to be misidentified in many Indian collections, with *O. mimica* nearly always wrongly identified as *O. sauzeti*, the more common and abundant species. Kapur's (1958) habitus and male genitalia figures for *O. sauzeti* were in fact those of *O. mimica*. It is also likely that molecular sequences for these two species could be based on wrong morphological identifications. This is illustrated by the fact that in the website of Barcode of Life Database (BOLD), the photograph of *O. sauzeti* is featured in the species page for *O. mimica* (from Pakistan).

We characterized the two species by their *cox1mtDNA* gene sequences and generated DNA barcodes as additional tools of diagnosis in conjunction with the already documented morphological characters. In this paper, we provide a complete illustrated account of the known morphological differences between the two species coupled with DNA barcodes, which should be useful in separating them.

MATERIALS AND METHODS

(i) Morphological studies

The examined specimens of *O. sauzeti* and *O. mimica* are deposited in the reference collections of the National Bureau of Agricultural Insect Resources, Bangalore. Photographs of morphological characters and genitalia were taken using a Leica M205A stereo microscope and composite images were generated from image stacks by Combine ZP software. The images were touched up for clarity and plates prepared in Photoshop Elements 11.

(ii) Amplification of *mtDNA COXI* gene and DNA barcoding

Card mounted specimens of *O. sauzeti* and *O. mimica* collected from Uttarakhand and Sikkim, respectively (<6 months old), were morphologically identified and used for DNA extraction and sequencing of 5' end of *cox1 mtDNA* (cytochrome c oxidase subunit 1 gene corresponding to the standard animal DNA barcoding locus) in the Molecular Entomology laboratory at NBAIR, Bangalore.

Genomic DNA was extracted from a single adult using QiagenDNeasy® kit, following the manufacturer's protocols. Specimens of both species were retained as voucher specimens after DNA extraction at NBAIR, Bengaluru. The DNA thus obtained was subjected to polymerase chain reaction (PCR) following standard protocol as described by Hebert *et al*. (2003). The following primers were used: forward primer (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198 5'-

TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in PCR tubes obtained from M/s Tarsons, Kolkata, India, following manufacturer's protocol, using de-ionized distilled water. The amplified products were analyzed on 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sequenced in an automated sequencer (ABI Prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) using primers both in forward and reverse directions.

Sequences obtained were checked for homology and frame shifts by using NCBI-BLAST and ORF finder. As no insertions, deletions or stop codons were observed in 2nd frame of DNA, sequences were chosen from ORF finder for submission to GenBank. The sequences were submitted to GenBank and the accession numbers obtained were uploaded to the project Agriculturally Important Insects of India (AGIPM) at Barcode of Life Database Systems (BOLD Systems, http://www.boldsystems.org) and DNA barcodes were generated under the following process IDs AGIMP of NBAIR, Bangalore.

RESULTS AND DISCUSSION

(i) Morphology

Oenopia sauzeti and *O. mimica* share the same overall external color scheme and the general pattern is superficially similar. They can be separated by the pronotal marking, elytral pattern, sculpturing on elytra and genitalia. Brief comparative diagnostic accounts of both species are given here with illustrations to facilitate easy identification based on external characters and male genitalia.

***Oenopia sauzeti* Mulsant (Figs. 1, 4, 6, 10–12)**
Oenopia sauzeti Mulsant, 1866: 281.
Oenopia sauzeti: Crotch, 1874: 158.-Kapur, 1963: 27.-Gordon, 1987: 19.-Yu, 2009: 100.
Gyrocaria sauzeti: Miyatake, 1967: 76; 1985: 15.-Poorani, 2002b: 103.

Diagnosis: Length: 3.40–4.60 mm. Ground colour (Fig. 1) of head and pronotum creamy yellow, elytral colour variable from creamy yellow to bright lemon yellow. Head black in female, yellow in male. Pronotum with a hat-shaped black marking (Fig. 4) on posterior margin, its posterolateral ends never reaching posterolateral corners of pronotum. Elytral pattern (Fig. 1) as illustrated, median sutural spot broad, distinctly transverse-quadrate and rectangular, occasionally with rounded edges. Elytral punctures distinct, interspaces between elytral punctures more or less smooth (Fig. 6) to alutaceous, without any microsculpture. Male genitalia (Figs 11, 12) diagnostic, with penis guide of tegmen deeply and narrowly parabolic (Fig. 11), penis (Fig. 12) with an elongate capsule having distinct arms.

Distribution: *Oenopia sauzeti* is distributed in India (Assam, Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Manipur, Meghalaya, Nagaland, Mizoram, Punjab, Sikkim, Tripura, West Bengal, Uttar Pradesh, Uttarakhand), Pakistan, Nepal, Bhutan, Myanmar, Thailand, Laos,

Vietnam, Taiwan and China. It is very common in all the northeastern states of India. In northern India, it appears to be more prevalent in higher elevations and cooler climes and rarely found in the plains. It was introduced in North America for controlling balsam woolly aphid [*Adelges piceae* (Ratzeburg)], but did not establish (Amman & Speers, 1964; Mitchell& Wright, 1967).

Hosts: It feeds mainly on aphids and also whiteflies. Agarwala and Ghosh (1988), Irshad (2001) and Poorani (2002) listed some of the common hosts of this species. Some of the common hosts documented are as follows: **Hemiptera**: **Adelgidae**: *Adelges* spp. on conifers. **Aphididae**: *Aphis gossypii* Glover, *A. craccivora* Koch, *A. pomi* De Geer, *A. spiraecola* Patch, *Acyrthosiphon pisum* (Harris), *Chaitophorus* sp., *Hyadaphis* sp., *Metopolophium dirhodum* (Walker) (as *Macrosiphum graminum* Theobald), *Sitobion rosaeiformis* (Das), *Myzus obtusirostris* David, Narayanan & Rajasingh, *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* L., *Sipha maydis* (Passerini), *Schizaphis graminum* (Rondani), *Sitobion avenae* (F.); *Sarucallis kahawaluokalani* (Kirkaldy)) (label data). **Aleyrodidae**: *Aleurolobus barodensis* (Maskell), *Neomaskellia andropogonis* Corbett, *Neomaskellia* sp. **Cicadellidae**: *Evacanthus repexus* Distant (Cicadellidae). **Acari**: *Tetranychus* sp.

***Oenopia mimica* Weise (Figs. 2, 3, 5, 7-9)**

Oenopia mimica Weise, 1902: 505.-Iablokoff-Khnzorian, 1979: 70 (as synonym of *O. sauzeti*).-Mader, 1935: 343.-Poorani, 2002b: 104.

Gyrocaria mimica: Miyatake, 1985: 16.
Oenopia sauzeti sensu Kapur, 1958: 331.

Diagnosis: Length: 3.0–4.3 mm, usually much smaller than *O. sauzeti*. Basic colour scheme (Fig. 2) similar to that of *O. sauzeti*, ground colour of head and pronotum creamy yellow, of elytra bright lemon yellow to creamy yellow. Head black in female, yellow in male. Pronotum with a black macula (Fig. 3) positioned on posterior margin similar to *O. sauzeti*, but its outer edges posteriorly extended, touching posterolateral corners of pronotum. Elytral pattern (Fig. 2) basically similar to that of *O. sauzeti*, except median sutural marking distinctly more elongate, gradually dilated and oval in the middle, and narrowed towards both ends. Elytral punctation (Fig. 5) distinctive, punctures somewhat finer, placed farther apart and slightly less dense compared to those in *O. sauzeti*, with conspicuous microsculpture in interspaces between elytral punctures. Male genitalia (Figs 8, 9) diagnostic, with penis guide of tegmen more widely emarginate and somewhat broadly v-shaped (Fig. 8), penis (Fig. 9) and penis capsule distinctly stouter.

The elytral pattern in *O. mimica* is also similar to that of *O. smetanai* Canepari (1997), another species distributed in the Nepal and Indian Himalayas. *Oenopia smetanai* is even rarer than *O. mimica* and can be distinguished from the latter by its much smaller size (only 2.8–3.0 mm long), pronotum with a pair of oblique oval median spots and the male genitalia (illustrated by Poorani, 2002).

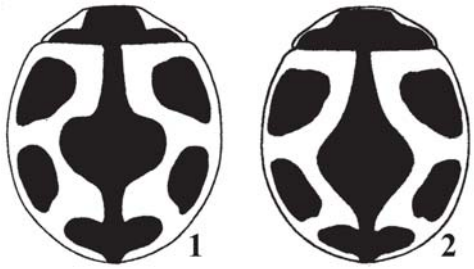


Fig. 1. *Oenopia sauzeti*: Adult, dorsal view; Fig. 2. *Oenopia mimica*: adult, dorsal view

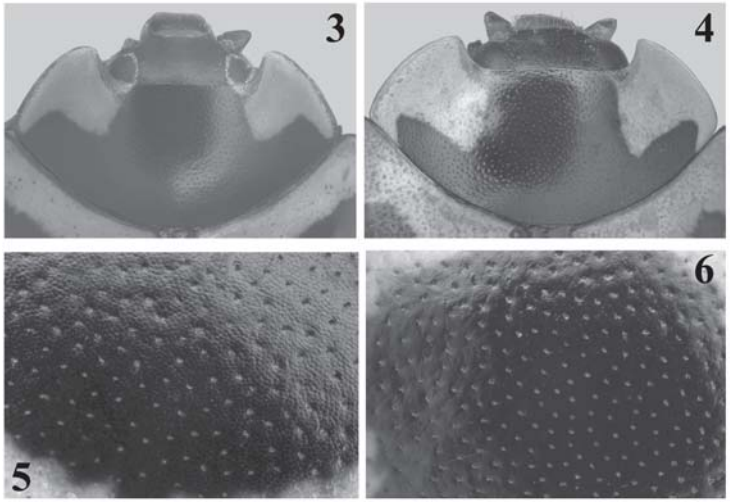
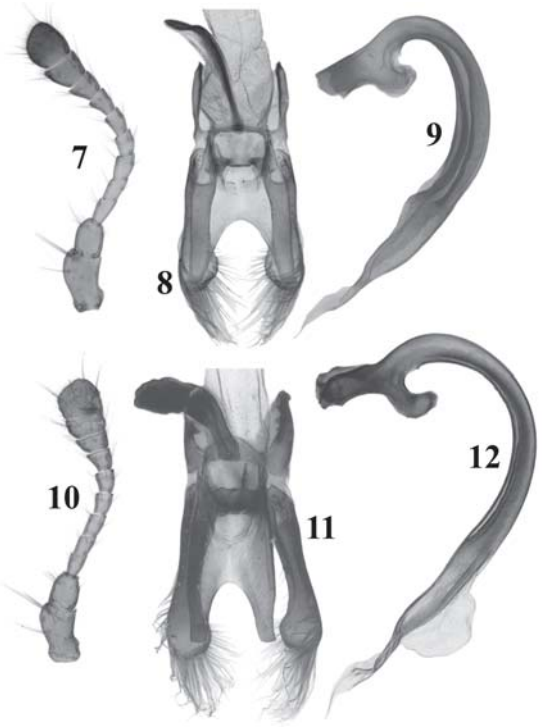


Fig. 3. Pronotal marking in *Oenopia mimica*; Fig. 4. Pronotal marking in *O. sauzeti*. Fig. 5. Elytral punctation in *O. mimica;* 6. Elytral punctation in *O. sauzeti.*

Distribution: *Oenopia mimica* is more or less confined to the upper reaches of Himalayas (Arunachal Pradesh, Himachal Pradesh, Sikkim, Uttar Pradesh) and is also known from Nepal, and Bhutan. There are some unconfirmed reports of its occurrence from Pakistan.

Hosts: *Oenopia mimica* is known to feed on *Adelges* spp. on silver fir, spruce and other coniferous vegetation; *Taoia indica* (Ghosh & Raychaudhuri) (label data). Host records from published literature are suspect and not included here.

Notes: Parts of Crotch's (1874) description of *Oenopia sauzeti* appear to match *O. mimica* better than *O. sauzeti.* His description of "thorax black, anterior angles with a quadrangular whitish spot, the inner angle produced to a point on the disc, outer portion prolonged to the posterior angle of the thorax" can be broadly applied to both species, but fits *O. mimica* more than *O. sauzeti.* Weise (1902) described *O. mimica* much later. It is not clear if the original type



Figs. 7–9. *Oenopia mimica*: 7. Antenna; 8–9. Male genitalia: 8. Tegmen, ventral view; 9. Penis; Figs 10–12. *Oenopia sauzeti*: 10. Antenna; 11–12. Male genitalia: 11. Tegmen, ventral view; 12. Penis.

series of *O. sauzeti* had any specimens of *O. mimica* also and Crotch's (1874) description is probably a result of his having examined more than one species in the material available to him. Gordon (1987) designated a lectotype for *O. sauzeti* (deposited at University of Cambridge, Crotch Collection), but did not mention anything about this.

Miyatake (1985) did not mention the difference in elytral sculpture between the two species, though it is the major distinguishing feature of *O. mimica*. The male genitalia are diagnostic for both species. The female genitalia in *O. sauzeti* and *O. mimica* are similar with the spermatheca differentiated into a distinct cornu, nodulus and ramus with a well-defined infundibulum, but the shape of the infundibulum is diagnostic for each species (see Poorani, 2012 for illustrations). Besides these characters, the antenna is also useful in separating the two species. In *O. sauzeti*, antennomeres 9 and 10 are distinctly transverse and the club is short and compact (Fig. 10). In *O. mimica*, antennomeres 9 and 10 are only slightly broader than long or nearly as broad as long (Fig. 7) and not transverse and the club is distinctly more elongate.

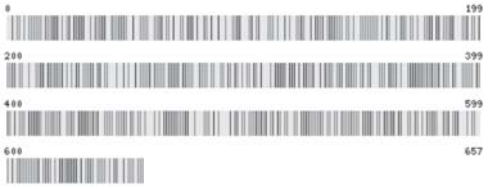
(ii) *cox1 mtDNA* **sequences and DNA barcodes**

The *cox1 mtDNA* gene sequence of 658 bp was obtained for both *O. sauzeti* and *O. mimica*. The *cox1 mtDNA* sequence of *O. sauzeti* from India had 98% similarity with that of another *O. sauzeti* isolate LBB41 (from China), confirming that they were conspecific. *Oenopia mimica* had 87% similarity with *Calvia quatuordecimguttata* (from Germany), an unrelated genus and species. The *cox1 mtDNA* sequences of *O. sauzeti* and *O. mimica* had only 89% similarity upon pair-wise alignment, which distinguished *O. sauzeti* from *O. mimica* with 75 nucleotide differences, thus confirming that these are distinct species.

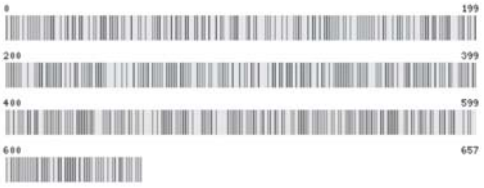
The GenBank accession numbers for the sequences of *O. sauzeti* and *O. mimica* were KR349051 and KR349052, respectively. Both the sequences were submitted to BOLDSYSTEMS and DNA barcodes obtained with accession numbers AGIMP042-15 for *O. sauzeti* and AGIMP043-15 for *O. mimica*. Species boundaries are established following a 2% divergence criterion (Hebert et al., 2003), based on the assumption that *cox1 mtDNA* divergences usually do not exceed a 2% divergence within a known species, whereas different species generally show a greater degree of divergence. Going by this criterion, the *cox1 mtDNA* sequences clearly separate *O. sauzeti* and *O. mimica*. Though the morphological differences between *O. sauzeti* and *O. mimica* are distinctive enough, these may be too subtle for the so called economic entomologists and the illustrations given here and the DNA barcodes should prove more useful for them in separating these two species.

DNA barcodes of *Oenopia sauzeti* and *O. mimica*

>*Oenopia sauzeti* _AGIMP042-15_ KR349051



>*Oenopia mimica* _AGIMP042-15_KR349052



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Short Communication No. ent. 40407

Rediscovery of the shieldbug *Menedemus vittatus*, with notes on *M. hieroglyphicus* (Heteroptera : Pentatomidae : Pentatominae: Sciocorini), from Pune, Maharashtra, India

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ABSTRACT: The first illustrated record of *Menedemus vittatus* (Dallas, 1851) is given after a span of more than 150 years since the original description and over 100 years since its redescription by Distant (1908). A note on mass mortality of *Menedemus hieroglyphicus* Distant, is added. Both species are perhaps endemic to India.

KEYWORDS: Pentatominae, Sciocorini, *Menedemus*

Distant (1899) erected the genus *Menedemus* to accommodate *Sciocoris vittatus* Dallas, 1851 as the type species (locality data 'Hab.?Africa') and in the same paper described *M. hieroglyphicus* as a new species (locality 'Bombay'). Later, Distant (1908: 436-437) recorded *M. vittatus* from 'Bombay, Bor Ghat (Dixon)' and here I record the species from Pune (Maharashtra State, India). These two species (probably endemic to India) are the only two species of the genus as a third species, *M. lewisi* Distant (1899), is now treated as *Sciocoris lewisi* (Distant), in David Rider's website (http://www.ndsu.nodak.edu/ndsu/rider/Pentatomoidea/, accessed July 2015).

The purpose of this note is to provide the first digital illustration of *M. vittatus*, record its recent collection in Pune and give brief redescription of the species. Comparative pictures of related species *M. hieroglyphicus* and *Sciocoris indicus* Dallas, 1851, all belonging to the tribe Sciocorini, are also provided. Images of the 'types' of both the species of *Menedemus*, obtained through the courtesy of Natural History Museum (NHM), London and Dr. Mick Webb, Curator of Hemiptera at NHM, are also included.

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M. hieroglyphicus has been found in many places and is apparently a common species all over south India. I have myself seen specimens from various parts of Maharashtra, and it is known from Karnataka and other parts of south India (Salini and Viraktamath 2015).

M. vittatus, however, seems to be a rare species. Except for its original description and the subsequent redescription by Distant, no one has reported this species. A recent checklist of Pentatomidae of south India (Salini and Viraktamath 2015) and an earlier survey of the Indian Pentatomidae (Azim 2011), based mainly on specimens at IARI, New Delhi, do not include this species.

Distant (1902) placed both *Sciocoris* Fallen and *Menedemus* in "Sciocoraria" (under Pentatomidae) citing Atkinson, with the following general characters, quoted verbatim: "head clypeated, not, or seldom, narrower than the base of the scutellum, foliaceously dilated; ocelli remote from the small eyes; antenniferous tubercles remote from the margins of the head, not distinguishable from above; basal joint of the antennae not reaching the apex of the head; scutellum more or less narrowed from the base; connexivum flattened, laminated". Now these genera are placed in the Tribe Sciocorini of the subfamily Pentatominae. The genera *Sciocoris* and *Menedemus* were separated only on the basis of the shape of scutellum by Distant (1902): thus in *Sciocoris* the scutellum is rather sharply narrowed while in *Menedemus* it is gradually so. *M. hieroglyphicus* and *M. vittatus* can be easily separated from each other as *M. vittatus* has broad, ochraceous bands on dorsal as well as ventral side whereas in *M. hieroglyphicus* there are only thin, broken ochraceous lines on dorsal side. In fact, while erecting the genus *Menedemus*, with *Sciocoris vittatus* as the type species, Distant (1899) had stated that *Menedemus* is allied to *Sciocoris* "but with the head a little longer and narrower and with the lateral margins distinctly reflexed" and it differs from *Sciocoris* in possessing distinctly "ornamental coloration of generally fasciate character". The prominent bands seen on dorsal side of *M. vittatus* are thus diagnostic. These facts can be seen in the images of all three species provided here (Figs 1 to 3). Even ventral view of the abdomen of all the three species shows that they can be separated easily on the basis of coloration and punctures on pregenital sternites as well as on the basis of partial view of the pygophore (Figs 4 to 6). Genitalia of male could not be dissected because of insufficient material, especially for *M. vittatus* and *S. indicus* and so comparison cannot be provided now but will be pursued subsequently. *M. vittatus* and *M. hieroglyphicus* are more or less of the same size (about 7 mm), females being slightly larger in *M. hieroglyphicus*; *S. indicus* is a small species (about 5 mm).

Redescription of *Menedemus vittatus* (Dallas, 1851: page 133).

Material Studied: 1 male and 1 female, coll: Shriraj, dead specimens found on the banks of a pond, old fort –Sinhagarh, Pune, Maharashtra State, India, in December 2013.

This bug has been briefly but adequately described, for identification purpose, by Dallas and later by Distant (as cited above), but not illustrated by anyone. Besides, these descriptions are quite old and not known to students / researchers working on biodiversity programs. Not

PLATE I: Figures 1 to 3 Dorsal habitus: 1) *M. vittatus* 2) *M. hieroglyphicus* 3) *Sciocoris indicus* Figures 4 to 6 Ventral view of abdomen 4) *M. vittatus* 5) *M. hieroglyphicus* 6) *Sciocoris indicus*

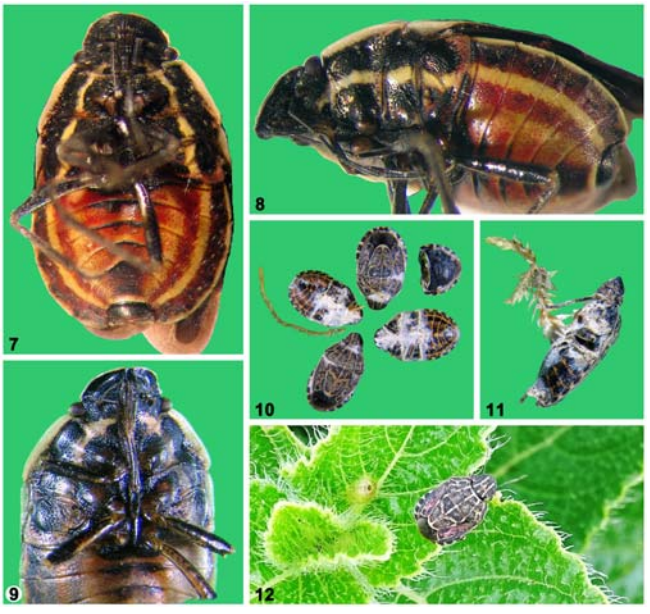

PLATE II: Figure 7. Full ventral view, *M. vittatus.* Figure 8. lateral view *M. vittatus.* Figure 9. Ventral view of thorax *M. vittatus.* Figure 10. *M. hieroglyphicus* affected by fungus. Figure 11. *M. hieroglyphicus* as above, entangled in moss. Figure 12. Live *M. hieroglyphicus* on *Strobilanthes*

PLATE III: Figure 13. Type, *M. vittatus.* Figure 14. Type, *M. hieroglyphicus*

only that, as remarked earlier, this bug has not been collected or reported in literature for 150 years from India (except by Distant). For this reason alone the salient features are redescribed and illustrated in this note.

Coloration: Dark brown to almost black bug of medium size (7.5 mm total length) with ochraceous longitudinal stripes on dorsal as well as ventral side. Dorsally three stripes on head that are continued onto pronotum and scutellum; in addition, lateral borders of pronotum and basal margin of corium also with stripes of same color. Red colored elongate patches, some of which are smooth without punctures, are seen on pronotum, scutellum and corium; membrane smoky with parallel veins (see Fig. 1). Ventrally head dark brown; antennae and labium brown; under surface of thorax dark brown, margins ochraceous; a broad, ochraceous band on either side. Abdominal sterna largely ochraceous and with reddish suffusion in median region with some black transverse lines or patches medially on anterior margins of some segments, with broad red band on lateral side which is flanked on outside by brown band beyond which abdominal margin is again narrowly ochraceous; genital capsule (pygophore) dark brown in male (Figs 7, 8).

Head: breadth (inclusive of eyes) more than length; apex rounded, clypeus (= median lobe of head) arrow like, shorter than mandibular plates (= lateral lobes of head), latter meet in front of clypeus; entire surface with dense, coarse punctures (except some parts of ochraceous stripes which are smooth); extreme border slightly reflexed, translucent and smooth. Eyes moderate, touching anterior border of pronotum; ocelli closer to eyes than to each other. Antennae slender, blackish, 5 segmented, first antennomere not reaching apex of head, 4th and 5th antennomeres with many fine black setae. Underside of head dark brown to black, with dense punctures, excepting ochraceous band. Labium just passing metacoxae (Fig. 9).

Thorax: Pronotum transverse, anterior margin sinuate, anterior angles gently rounded, as broad as head at level of eyes, lateral margins straight, slightly reflexed and smooth, humeral angles rounded, posterior margin straight over scutellum; entire surface with dense, coarse punctures except ochraceous bands which possess sparse punctures; calli not prominent, smooth. Sternum with dense, coarse punctures, punctures sparse in ochraceous bands.

Scutellum tongue like, rounded at tip, lateral borders straight, basal corners depressed, slightly convex dorsally, punctures coarse and thick all over except for ochraceous bands, with some areas between ochraceous bands smooth and reddish.

Hemelytra: Corium with coarse punctures, less dense than elsewhere dorsally, with only outer angles extending beyond scutellum; membrane extending beyond abdomen, smoky with four parallel veins.

Abdomen: punctures sparse, not coarse.

***Menedemus hieroglyphicus* Distant, 1899: 430**

This bug was redescribed and illustrated by Distant (1902: 127-8) and is commonly observed hence only digital illustrations are provided here (Figs 2, 5). It is also well known and perhaps more widely distributed species. Several specimens were studied. Many were found dead in drying moss that grows profusely on tree trunks during monsoon (locality and date of observations are the same as for *M. vittatus*). Many bugs were still intact but the other specimens were partially decayed and covered with white fungus (Fig.10, 11) but were easy to identify as these bugs have characteristic pattern of ochraceous lines on dorsal side. It is surprising why large number of bugs (we counted in excess of 50 at one place on Sinhagarh) were in such condition. I do not know the host plant of this species, in Sinhagarh area, in Pune. On Kas Plateau, in Satara, Siddharth Kulkarni (personal communication, with photographs) observed it in good numbers on a species of *Eriocaulon* as well as *Strobilanthes* (Fig.12).

It is my pleasure to include here the images of the types of both the species which are very well kept at the Natural History Museum, London. The images of *M. vittatus* (Fig. 13) and *M. hieroglyphicus* (Fig. 14), with label data, support this note by confirming the identity of these two bugs.

Why bugs are attracted to moss and what is the cause of their death is uncertain at present. Similar incidence was observed by my students in Amboli Ghat area, during September 2014, but here at least a few live bugs were still seen in moss. Additional surveys and identity of fungus (entomophagous?) may help to solve this problem of mass mortality of bugs in moss during late monsoon.

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I am grateful to Drs Max Barclay and Mick Webb of Natural History Museum (NHM), London, for providing images of the types of both species and for permitting me to use these images here; the copyright of these images rests with NHM, London. This helped to settle the identity of *Menedemus vittatus* beyond doubt. Dr. Mick Webb also made some useful suggestions to improve this note. Dr. David Redei (Nankai University, China) also has helped in various ways in our work on Heteroptera. I also thank Derek Croucher for preparing excellent photographs of these types at NHM and Siddharth Kulkarni for photos of live bugs on plants *Eriocaulon* as well as *Strobilanthes*, from Satara. Thanks to Dr. Mandar Datar for identity of plants. Sameer Padhye, Mihir Kulkarni, Sayali Sheth and Shruti Paripatyadar helped in locating these bugs in moss and all helped me in field work; Shriraj also prepared plates for this note. Finally I am grateful to the authorities of Modern College for facilities and encouragement.

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Parasitisation of soft brown scale, *Coccus hesperidum* Howard by an aphelinid wasp, *Coccophagus ceroplastae* (Howard) infesting orchids from Sikkim, India

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ABSTRACT: *Coccophagus ceroplastae* (Howard) an aphelinid parasitic wasp was found to parasitize populations of the soft brown scale, *Coccus hesperidum* Howard infesting the orchids in Sikkim, India. Parasitization under natural conditions was found to range from 5-45%. This is the first report of *Coccophagus ceroplastae* (Howard) on Soft brown scales on orchids. © 2015 Association for Advancement of Entomology

KEYWORDS: *Coccophagus ceroplastae, endoparasitoids, Coccoidea, Coccus hesperidum,* Orchids

Orchids are one of God's beautiful creations and have since long been admired by man. Orchidaceae, one of the largest families of flowering plants is no exception to this richness of variety. India alone has contributed nearly 1150 species belonging to 164 genera and many are discovered year after year. Knowing the immense value of orchid, collections from different parts of the country are maintained in the germplasm at National Research Center for Orchids, Pakyong, Sikkim. The place is situated at the elevation of 1300m between 27^0 4"- 28^0 7'48" N and 88^0 58"-88^0 5' 25" E longitude experiences average maximum temperature 17-28^{0}C and minimum 6-20^{0}C, maximum relative humidity 81-95 % and minimum 43-73%. Pakyong receives an annual rainfall upto 300 cm. The orchid flowers are well known for their uniqueness in shape, size, colour and scent are exquisitely attractive, normally remain fresh for longer period of time in comparison to other flowers. At our centre the plants are kept under open poly house/partial shade. Under poly house conditions the orchids are subjected to infestations by several insects and non-insects pests.

Soft brown scale, *Coccus hesperidum* belonging to Coccoidea, infests orchids. The adults

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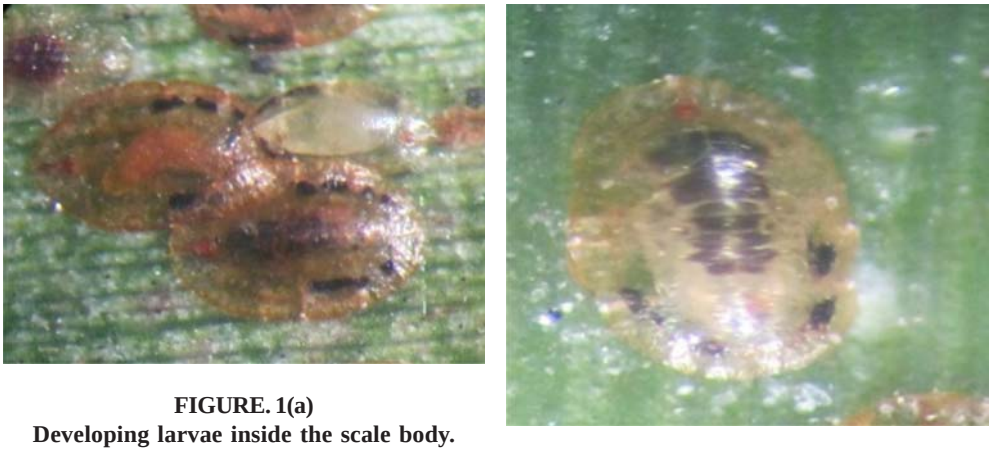

FIGURE. 1(a)
Developing larvae inside the scale body.

FIGURE.2.Parasitized&healthyscales

are pale brown to yellowish in colour mottled with brown spots. Direct injury by this insect pest is caused by sucking the cell sap from the leaves, petioles, leaf sheaths and pseudobulbs of many orchid genera. Being soft scale, indirect injury is caused by secretion of honeydew; a clear sticky liquid that serves as a medium for growth of black sooty molds thus hampering the photosynthetic activity of the plant. The plant losses its vigour and the overall health of the plant are lost.

The aphelinid wasp, *Coccophagus ceroplastae* (Howard) was found to parasitize the soft brown scales especially the younger stages. They are endoparasitoids of coccoidea mainly soft scales, and some are parasitic on mealy bugs. The females of this wasp deposits egg into the body of the scales and the larval and pupal development took place within the body (Fig.1 a & b). The scales with developing larvae and pupa inside them were darker in colour due to parasitism (Fig. 2).The adults black in colour emerged by making a circular hole on the scale cover (Fig. 3). The percent parasitization of the soft brown scales under open poly house conditions ranged from 5-45%.

Coccophagus ceroplastae (Howard) was for the first time reported from *C. hesperidum* infesting papaya from Hyderabad, India by Joshi *et al.* in 1981. It has also been reported from Mango scale, *Chloropulvinaria polygonata* (Dinesh and Sinha, 1991). It was reported on *C. hesperidum* on Cardamom from Saklespur, Karnataka, India. From Darjeeling district of West Bengal, *Coccophagus ceroplastae* was reported to parasitized more than one species of scale insects (i.e. *Saissetia coffeae* and *Ceroplastes floridensis*) infesting Citrus (Konar and Roy, 2008). Studies revealed *Coccophagus ceroplastae* as parasitoid of soft scales such as *Coccus viridis, C. hesperidum, Saissetia coffeae, Ceroplastes* spp., *Pulvinaria psidii, P. polygonata* (Coccidae) (Hayat, 1998). In Bangladesh the aphelinid parasite *Aneristus ceroplastae* Howard was recorded from soft scales *Ceroplastes pseudoceriferus* Green and *Chloropulvinaria polygonata* (Ckll.) (Homoptera: Coccidae) on mango (Ali M, 1978). *C. ceroplastae* parasitized *Aspidiostus destructor* S. infesting Mango in U.P, India. In Australia *C. ceroplastae* was reported to be the dominant parasitoid in reducing the soft scale insect *Pulvinaria urbicola* (Cockerell) infesting *Pisonia grandis* trees (Smith *et al*. 2004). Huang and Huang (1988) reported *C. ceroplastae* from citrus scale, *Ceroplastes floridensis* in China. Hayat *et al*. (2003) reported *C. ceroplastae* from coccoids infesting sandalwood from South India.

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